culms.'' Schauer (40) writes that during 1921 in Baden, Germany, 30 per cent of the cereals were attacked by Dilophospora.

In the cases observed by the writer there was a decrease in yield in one of the fields of not less than 60 per cent. In full agreement with Kessler the writer observed that while only about 10 per cent of the heads of fully grown plants were infected, there were to be seen, upon closer examination of the fields, a much larger percentage of small and stunted plants, the heads of which were completely destroyed by the fungus. Such plants are usually overlooked by a superficial examination of the fields.

The actual loss due to this disease alone, however, will always be a very difficult matter to estimate, since the disease, as already stated, occurs always together with the nematode disease of the cereal crops. It is certain that this disease is much more injurious than the nematode disease and usually completes the destruction of the plants which are only slightly and partially injured by the nematodes. Losses of as much as 40 to 50 and even more per cent by actual count, have been reported for *Tylenchus tritici* (3, 4). A combination of both disases under favorable conditions may therefore completely ruin the crop. In some of the writer's experiments the infected plots failed to form any heads whatever, while in others the few heads formed yielded mostly nematode galls.

DESCRIPTION OF THE DISEASE

The symptoms of the disease, as a whole, are so pronounced and singular that it can never be mistaken for other diseases.

Since this disease occurs only on plants attacked by *Tylenchus tritici* knowing the symptoms of the nematode disease in pure form will help us to distinguish the symptoms of the Dilophospora disease and to understand how the symptoms of the two diseases in combination are brought about.

The Nematode Disease

The nematode disease usually becomes apparent by its effect on the heads of the plants, although symptoms of the disease may occur on all parts of the plant above the ground. Under normal conditions the young plants show the first symptoms when in the third or fourth leaf. If the weather is not too cold, symptoms appear sometimes only 15–20 days after sowing. The infected plants can be recognized by the decided wrinkling, rolling and distortion of their upper leaves and the very pronounced swelling of their bases. Such plants do not stand upright; at least at first, they are usually inclined or slightly curved. Their leaves are shorter and wider; the leaf sheaths are also wider, somewhat swollen with strongly phied mesophyll tissues, they are brittle and stand loose around the leaf sheaths, at first at least, it forms no pycnidia, but spreads in the concavity of the sheaths and the enclosed young leaves, thus firmly binding together the upper central portions of the plant. The growing point of the plant thus firmly shut off on all sides, and still free from any fungus infection, but commonly surrounded by numerous nematode larvae, can never break through the leaf sheaths thus bound together and eventually

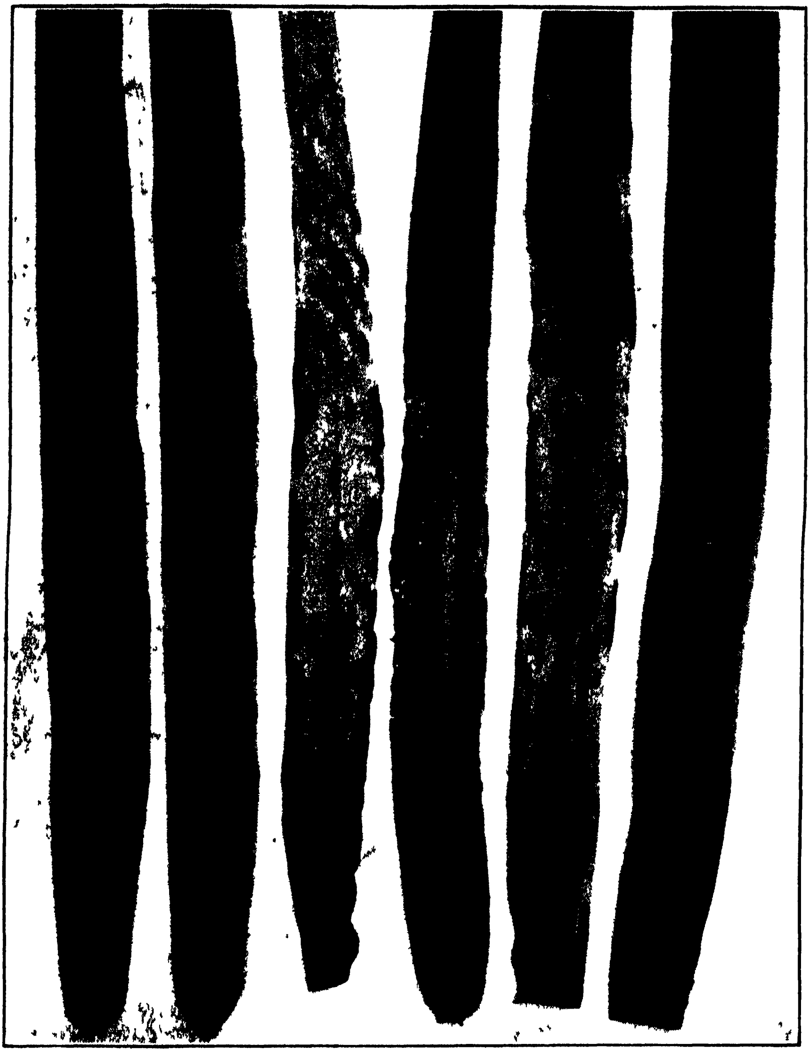

FIG. 2. Wheat leaves attacked by *Dilophospora alopecuri.* The blotches and stripes on them consist of pycnidia.

becomes a prey to the downward advancing fungus growth. In some cases the growing point pushing upwards against the bound together and partially or wholly killed upper leaves and leaf sheaths, and not being able to open a way begins to curl under its own pressure, filling up the cavity in which it finds itself firmly enclosed and forming a kind of knot (Fig. 1). Externally the upper portions of such plants resemble a cigar and have a spindle or conical shape. Often the increasing knot may succeed in breaking through the leaf sheaths, but its top, which by this time is also invaded by the fungus and firmly attached to the surrounding tissues, remains within the sheath cavity. Plants so effected remain very small, fail to head out, and die quite early in the season. By far the largest number of infected plants belongs to this group. Some of the plants thus killed send out tillers from their bases, which usually remain free from the disease and develop normally, but ripen much later than the normal plants.

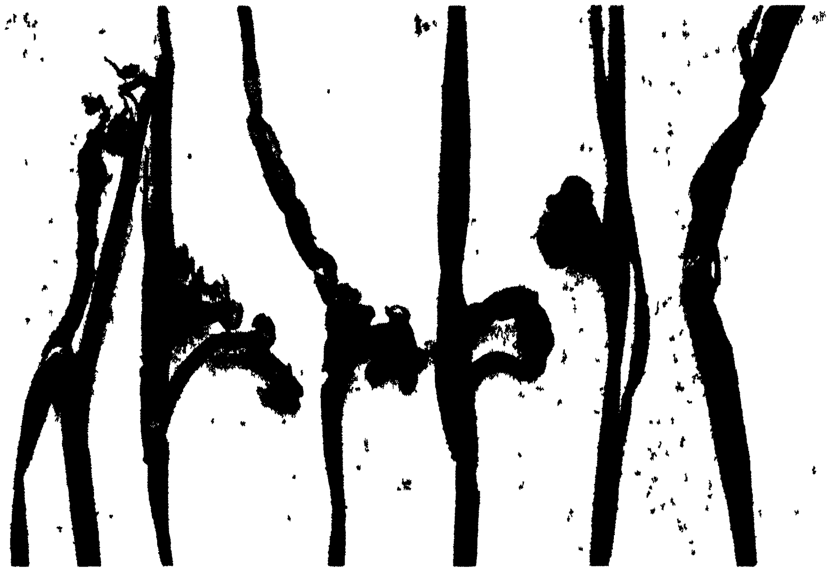

FIG. 3. Upper portions of fully grown wheat plants showing effects of *Dilophospora alopecuri* infection. The plants are deformed due to the gluing together of the young heads to the sheaths of the upper leaves.

Plants on which the symptoms of the fungus infection appear somewhat later and after the formation of the second or third node, may also be deformed and killed in the same way as the above plants. This group of plants is the next largest. Where the symptoms of the disease appear shortly before the heading out of the plants the infected heads of the otherwise normal plants may remain attached to the upper leaf sheath to which they are bound by the fungus growth (Fig. 3). Similar deformation of cereal plants can be brought about also by bacterial diseases (47). The kind of plants, however, which first attract attention upon a superficial examination of infected fields, are the otherwise perfectly normal plants, the heads of which are free but wholly or partially deformed and destroyed by the fungus. These are the plants least injured and last attacked by the fungus. The deformed heads look as if they had been pinched up into a point when young and afterwards charred. The deformation of the heads may be partial, as already mentioned, the top being usually involved, or it may include the whole head (Fig. 4). Superficially the affected areas at first are white, being covered by a dirty, white, fungus growth, which later on becomes dark brown to black, resembling somewhat a sclerotium. The deformed head portions internally consist of a white mycelial tissue in which are embedded the glumes and rachis. The normal portions of the partially deformed heads are usually full of nematode galls, but they may in some cases form normal kernels.

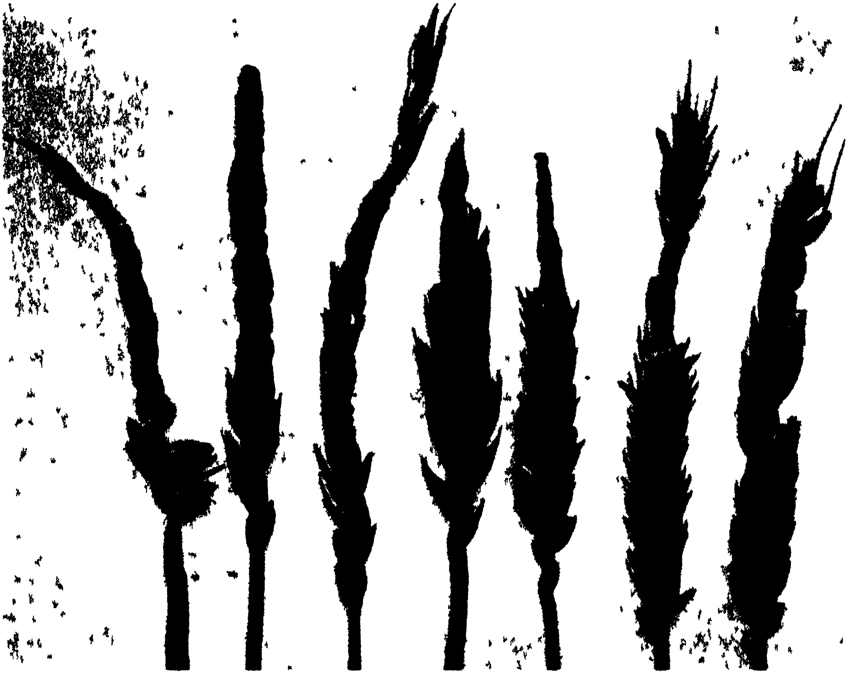

FIG. 4. Wheat heads showing various degrees of *Dilophospora alopecuri* infection. Infected portions are charred in appearance.

The underground portions and bases of all infected plants are absolutely free from any pathologic changes and resemble in every respect those of the healthy plants.

DESCRIPTION OF THE CAUSAL ORGANISM

Taxonomy

Fries (9) in 1828 was the first to describe the fungus causing this disease under the name of *Sphaeria alopecuri.* Duby (7) described it several years later under the same name. In 1840 Desmazières (5), to whom as well as to Fries and Duby the original collector, Guepin, had sent part of his material, established the new genus Dilophospora[1] for this fungus and applied the specific name *D. graminis,* citing *Sphaeria alopecuri* Fr. as a synonym. Fries (10) in 1849 accepts Desmazières generic name, but insists upon his own specific name. The name of the fungus therefore should be *Dilophospora alopecuri* (Fr.) Fr. as Bessey (2) correctly pointed out. Fuckel (12) in 1861 described a similar fungus on *Holcus lanatus* under the name of *Dilophospora Holci* Fuckel, but changed this name in 1869 (13) to *Dilophospora graminis* forma *Holci* Fuckel. Fuckel and after him Saccardo (39), Ludwig (23, p. 318), Diedicke (6), etc., have described also an ascus and a conidal form of this fungus. So far the writer has never seen anything on the infected plants and artificial cultures of the fungus that could be taken for Dilophia or Mastigospora, the alleged ascus and conidial forms of Dilophospora. Störmer (50), Fron (11), etc., have expressed the same opinion.

Diagnosis

The diagnosis of the fungus given below is based upon the material gathered in the vicinity of Epen on wheat, rye and spelt, and upon artificial cultures and subsequent artificial infections made from the original material. A more extensive study of the fungus under different climatic conditions and on its various hosts may show that there are more than one Dilophospora species concerned with this disease.

The closely packed pycnidia, surrounded by numerous mycelia which are thick, short and rich in fat vacuoles, when examined with the lens, or in crossection under the microscope, seem to be embedded in a black stroma-like tissue as has been claimed by some mycologists. The pycnidia of this fungus however, as is shown by figure 5, lie completely free in the mesophyll of the leaves. This can be easily seen on leaves which have been decolorized by boiling in alcohol. On the leaf sheaths the conidia are formed in rows between the veins, quite uniformly scattered over the infected area, usually scattered but sometimes joined together in twos or threes.

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[1] The name Dilophospora is composed of dis = double, lophos = comb, and spora = spore.

The pycnidia are globose or elliptical, of a dark brown parenchymatous tissue, varying considerably in size, 120 to 300 μ, averaging 200 μ in diameter, thin walled, wholly immersed in the mesophyll of the leaf or leaf sheath, only the short papilla-like ostioles coming to the surface of the epidermis; pores 18–20 μ wide.

The spores are cylindrical, usually of same thickness throughout their length, only at the ends being somewhat constricted and ending in a number of hair or bristle-like appendages; the number of the appendages varies from three to six and more. The bristles may be simple or branched and may come from one point in the form of a tuft or may appear subsequently on an axis, resembling somewhat the main branches of a tree. Spores usually are not distinctly septate. Distinct septation becomes apparent in the germinating spores (Fig. 6). In the fully developed young spores there are usually to be seen a number of small dense bodies of uniform size and very evenly distributed in one line over the length of the spores, sometimes they are in pairs. In mass the spores are light yellow in color. Exclusive of appendages the spores measure 8.5 to 15 by 1.6 to 2.5 μ, averaging about 12 × 2.2 μ. The appendages are 5 to 7, rarely 10 μ long and about 0.5 μ thick at the base.

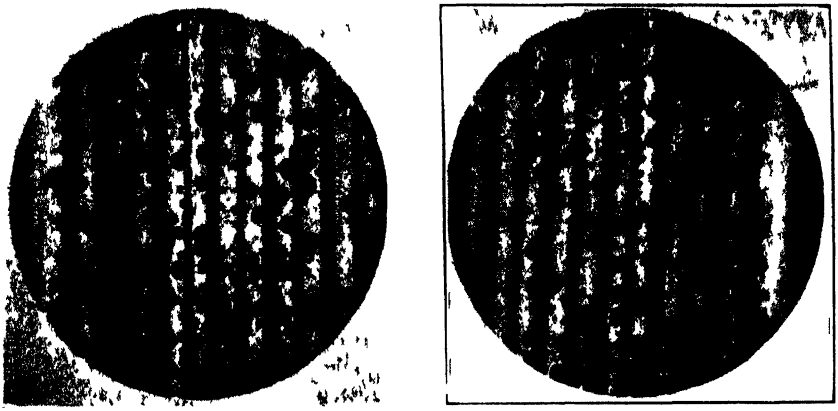

FIG. 5. Photomicrographs of portions of wheat leaves attacked by *Dilophospora alopecuri*, showing the pycnidia of the fungus. (Left) Upper side of leaf, the ostioles of some pycnidia are to be seen. (Right) Lower side of leaf. × 10.

PHYSIOLOGY OF THE CAUSAL ORGANISM

Cultural Characteristics

In general this fungus is a slow growing organism. Even under most favorable conditions it takes over a month to cover the surface of a slant culture. In the same length of time, in Petri dishes, it forms colonies of not more than 5–6 cm. in diameter. The fungus grows and fructifies best on rich, unfiltered hard oatmeal agar. Agar containing the whole content of the hulled oat kernels is much better than oatmeal extract. On the latter the colonies never attain any size and may fail to form pycnidia. Rich unfiltered, medium hard, cornmeal agar is the second best medium. The fungus grows and fructifies moderately also on plum decoction agar, malt agar, malt gelatine, carrot plugs, and potato agar, the last being the least suitable medium for this organism.

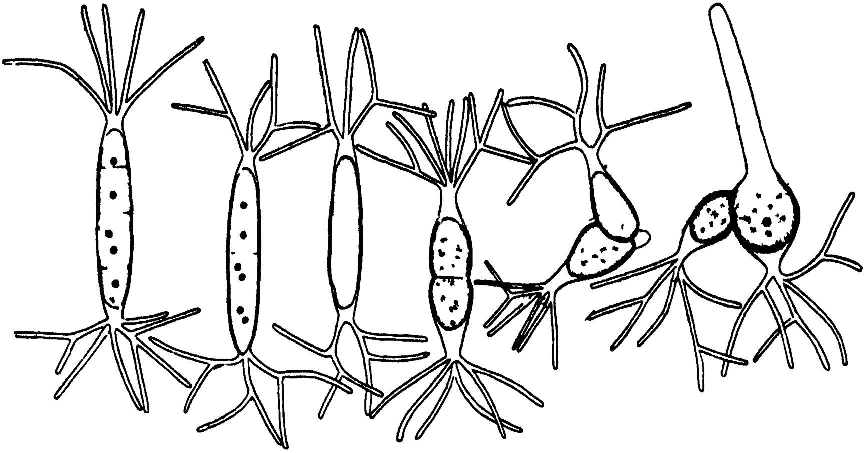

FIG. 6. Pycnospores of *Dilophospora alopecuri*. The three spores to the right represent various stages of spore germination. × 2000.

Mycelial growth is slow, at first only on the surface and quite loose, later and especially with the drying out of the culture there appears, in the cases where mycelia are used for transferring, a rick aerial growth, often forming large, irregularly round, comparatively dense tufts of mycelia. The aerial growth is in most cases, at first white or very slightly pink and may remain so for a long time; later the original growth takes a slightly grayish green to olive green color, which is primarily due to the formation of numerous pycnidia. The mycelium is of uniform thickness, sparingly branched and septated. With the appearance of pycnidia the aerial growth at the center of the colony disappears, the latter assuming a dark, olive green to black color. The aerial growth is scanty and fails completely when spores are used for transferring. On rich and favorable media the fungus forms concentric rings of pycnidia, especially in Petri dishes. Pycnidia are usually formed on aerial mycelia or on the layer of mycelia lying on the surface of the medium, but may also be formed in the substratum, especially along the edges. On oat and corn meal agar when pycnospores are used for transferring, pycnidia are formed in abundance and almost without any mycelial growth, just as is the case on the infected leaves.

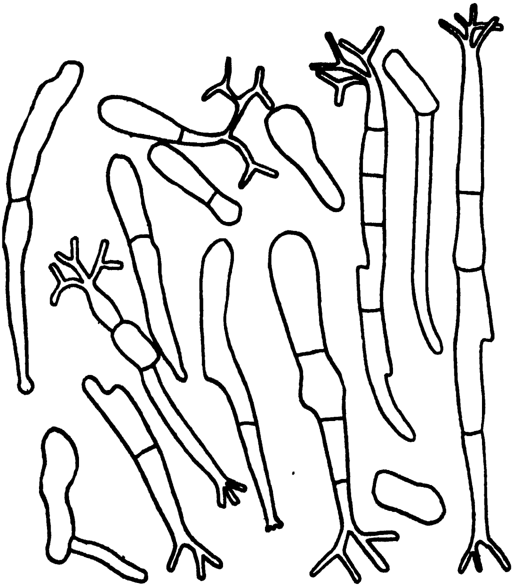

FIG. 7. Secondary spores of *Dilophospora alopecuri*. × 1000.

Spore Germination

The spores seldom germinate in water drops and not before the second or third day. On agar media they germinate in a shorter time and in much larger numbers. The germinating of the spores is preceded by a thickening of the cell walls and swelling of the middle portion of the spores; at the same time there appears a septum, dividing the spore in two parts. The swelling of the spores continues until they may reach twice their normal thickness. The swollen spore splits partially at the middle, so that the two halves stand to each other at an angle of from 60° to 45°. From the point of splitting there emerges a germtube (Fig. 6), which before reaching a length of 20–30 μ, forms one or more side branches. The end of the germtube stops growing further but sends out a number of shorter or longer appendages as seen on the spores and becomes itself a "secondary" spore. This also happens with the ends of the side branches. The young colony continues forming secondary spores for a number of days. As a result there becomes apparent on the agar surface a small, light cream or pink colored colony, resembling in every respect a bacterial colony. A microscopic examination shows that what was originally a single spore now represents a pile of sporelike bodies, in most cases attached to some other sporelike body (Fig. 7). From this bacteria-like colony there appear afterwards in all directions mycelia, thus giving rise to a fungus colony. On Petri dishes the bacteria-like colonies, especially if numerous, may persist for a long time and even until the drying out of the plate. (Fig. 8.)

FIG. 8. 35 day old culture of *Dilophospora alopecuri* on oatmeal agar. 4/5 natural size.

Not all spores germinate in the way described above. In some cases the spore instead of becoming thicker begins to grow in length, the appendages of the one end disappear and it takes the form and the function of the germ tube. In still other cases only one-half of the spore swells, splits partially from the second half, from which point appears the germ tube, while the other half seems to undergo no changes; or the protoplasm from it may pass into the first half. In still other cases the appendages of a spore disappear at one end, from which point then appears the germ tube without being preceded by an elongation of the spore itself. In some cases after the swelling and partial splitting of the spore there come germtubes from both halves of the spore. In such cases the two spore halves break completely away from each other.

METHODS OF INFECTION

Infection Material

Abundant spore material of *Dilophospora alopecuri* for infection work can be obtained by growing the fungus on boiled wheat, barley or unhulled spelt. Steaming of the media in Erlenmeyer flasks each time for half an hour on three consecutive days is sufficient to sterilize it. The sterilization of the media should be so regulated as to prevent the splitting of the kernels and the formation of a compact mass of the media. There should be added just as much water to the flasks as the kernels will be able to absorb during the sterilization; the media should be porus. The greater the porosity, *i.e.*, the surface of the media, the greater the number of pycnidia will be.

Heavy spore suspensions can be obtained by macerating thoroughly the content of a flask or culture tube in a mortar, transfer then to water, shake up thoroughly and filter through a fine cloth or metallic sieve, let the starch settle for 10 minutes, and decant. The liquid so obtained contains millions of spores and can be used for infection experiments or for mixing with nematode suspensions.

The length of infection experiments can be shortened very much and positive results can be secured in all cases by using nematode suspensions instead of nematode galls. Under natural conditions the nematodes leave the gall only after the rotting away of its thick walls, whereas if the nematodes are freed from the galls by opening them, they can directly attack the plants. For securing a nematode suspension the galls are placed in a small cheese cloth bag, the bag is then immersed in water in a small beaker and allowed to stand for 12 hours. Care should be taken to keep the bag under the water, otherwise the galls will soon be overgrown by molds. The galls thus softened are then torn into pieces by means of needles and left to stand in the water for 12 more hours, to give the nematodes a chance to leave the galls. The suspension of nematodes then is poured through a fine metallic sieve to separate the gall fragments from the suspension. Such a suspension of nematodes contains also a great number of bacteria, fungous spores and protozoa. To a considerable degree the nematode suspension can be freed from these contaminations by diluting the suspension with water and leaving it to stand in a large Erlenmeyer flask until the nematodes settle to the bottom. The water then can be drawn out by a siphon. This should be repeated several times.

It is much more difficult to free the nematodes and nematode galls from the Dilophospora conidia. All nematode galls which the writer had at his disposal were obtained from a sample of grain which was simultaneously infected also by Dilophospora, so that both diseases appeared even in infection experiments where only nematodes were used. The writer was able to obtain nematodes comparatively spore free by selecting only large, uninjured, shining, black galls that were free from fungus mycelia. Such galls were rinsed several times with sterile water, disinfected for 4 minutes in 1 per cent $MgCl_2$ solution, left to stand in distilled water for 4 hours, then torn open, freed from the nematodes, and removed at once from the water in which the nematodes remain.

Soil Infections

Quick and reliable infections with the nematode or Dilophospora disease can be obtained, if only the weather is warm, by infecting the soil with water suspensions of nematodes and Dilophospora spores. This can be done by infecting the soil at the time of sowing or after the emergence of the plants from the soil. For this purpose the writer made use in most cases of large pots. Pot infections have the advantage that the plants can easily be removed from one place to another; besides this the infection material remains limited to the pots. When the infection material is added to the pots at the time of planting, it is poured over the seed in the pots, the seed then is covered with a 2–3 cm. layer of fine, moist soil. In the cases where the infection material is applied to the young plants it is poured uniformly over them; over the plants is then sifted moist, fine soil so as to cover the surface of the soil on which the nematodes and fungus spores have been deposited. This is done to prevent an eventual drying out of the surface of the pots before the nematodes have had time to penetrate the soil or the plants.

All infected pots were kept constantly moist, because a drying out of the soil will naturally lessen the activity of the nematodes and may even delay or prevent the appearance of both diseases.

Wound Infections

If wound infections are ever to succeed they should be made at the vegetative point of the plants, though so far they have given no positive results.

The growing points of the plants can easily be located after the appearance of the first node. It stands about 1½ cm. above the uppermost node. The upper node, be it the second, third or fourth, can easily be located by feeling the culm of the plant between one's fingers. It lies deep under the several layers of leaf sheaths. Directly above it stands a still incompletely differentiated node followed by the vegetative point or rudimentary head of the plant. The two latter as already stated stand at a distance of about 1½ to 2 cm. above the well developed upper node. After locating the vegetative point, a split is made through the leaf sheaths and a drop of a heavy spore or nematode suspension is placed in the wound. The wound is then tied up. Inoculation of the growing point can be accomplished much easier by injecting the spore or nematode suspension into the central cavity of the plant near the vegetative point. For this purpose the syringes used by medical men may be used.

PATHOGENICITY OF CAUSAL ORGANISM AND ITS RELATION TO TYLENCHUS TRITICI (STEINBUCH) BASTIAN

All of the several hundred wheat, spelt, and rye heads infected with Dilophospora, which the writer gathered in the province of Limburg during the summers of 1923 and 1924, upon a careful examination proved to be attacked also by *Tylenchus tritici,* and had numerous nematode galls in the portions that were free from the fungus. In the subsequent experimental work and field studies on this disease all Dilophospora infected plants showed also the presence of nematodes.

The same condition has been observed for the first time in a single case by Richon (34) in 1882. Störmer (50) in 1904 reports that without any exception all of the spelt plants sent to him from Pfalz were attacked simultaneously by *Dilophospora graminis* and *Tylenchus tritici.* Störmer also made the suggestion that probably the fungus, which commonly occurs on grasses, being not so well adapted to spelt, could get foothold only on those plants that are weakened by the attacks of nematodes. He observed that not all of the plants infected with nematodes were also attacked by the fungus. Bessey (2) found in 1906 nematode galls and Dilophospora pycnidia on the same leaves of *Calamagrostis canadensis.* Fron (11) in 1912 on the other hand reported that not all Dilophospora infected heads carry nematode galls and thought that the occurrence of the two parasites on the same plants may be only a coincidence. Stebler (49) *et al.* observed in 1917 in one case the occurrence of Dilophospora and Tylenchus on the same wheat and spelt plants and wrote that "Such a simultaneous occurrence of the two diseases is nothing unusual, especially on spelt." Pape (30) in 1921 also reported that a portion of the Dilophospora infected wheat heads sent to the Biologischen Reichsanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, both from Baden and the Rhine province were also attacked by *Tylenchus tritici.* It may not be out of place to mention here that Maire (24), Marcinowski (25, pp. 67–116), and others have reported the simultaneous occurrence also of stinking smut, *Tilletia tritici* and *Tylenchus tritici.*

Though none of the above workers has suspected the existence of an intimate relation between these two diseases and while the up-to-date observations on the simultaneous occurrence of the two diseases on the same plants do not throw any light on the nature of this phenomenon, they nevertheless indicate that there must exist some relation between the two pathogenes.

It is well known that *Tylenchus tritici,* whose life history and parasitism has been extensively studied, is a true and highly specialized parasite (3, 4, 25), which can attack the susceptible hosts without the assistance of any external agents and in the absence of Dilophospora. Whether this applies also to *Dilophospora alopecuri,* which has never been studied, is not known.

At the hand of our present knowledge of the two parasites and the diseases caused by them it can be taken *a priori* as probable that this co-parasitism is not only of no use but even harmful for *T. tritici,* since it is evident that the nematodes can never form galls on the plants killed by the fungus or on the portions of the heads attacked by them, or if they should succeed to form any galls in these portions, the galls are overgrown and subsequently destroyed by the fungus.

Just how and whether this co-parasitism is beneficial to the second parasite will be shown further below.

Before doing this and in order to properly comprehend the way in which these organisms influence one another it will be necessary to review briefly the life history of *Tylenchus tritici.*

As is well known the above nematode forms numerous galls in the heads and sometimes also on the leaves of the infected plants. These galls, in each of which are present from 10 to 15 thousand living nematode larvae, fall on the ground during the harvesting of the plants or are carried into the soil with the seed. They can find their way back to the fields also by means of the manure, straw, animals, etc. Once in the soil and in the presence of moisture, the outer layer of the galls begins to rot or if injured in one or other way, their content is liberated. In the dry galls the nematodes are motionless and show no symptoms of life. In this condition they can survive twenty years. As soon as the gall and gall contents are moistened the nematodes become active and leave the gall shell, if this has been already ruptured or rotted away. Once free the nematodes move towards the nearest host plants and take a position in the concavity between the external and internal parts of the leaf sheaths of the young cereal plants. From here they advance further and further towards the center of the plant, moving always between the leaves until they reach the center, *i. e.,* the growing point of the plant, where they remain permanently as ectoparasites and where they are found in large numbers. With the growing of the plant they are pushed passively upwards by the growing point. As soon as the heads become differentiated and long before the heading out of the plants, the nematodes bore into the newly formed ovaries or around their bases.

The wounding and further stimulation of the host cells give rise to the galls, which are quite large and easily distinguishable when the heads emerge from the last leaf sheath. Usually a small number of nematodes are found in each newly formed gall; here they attain their full development and become sexually ripe, some male, others female. They then pair and lay a great number of eggs. From the eggs are soon hatched the young larvae, which after a short period of growing and with the ripening of the plants and drying of the galls become inactive, thus completing their life cycle.

In the writer's observations and infection experiments the Dilophospora disease, as already stated, appeared only on plants simultaneously attacked by the nematode disease.

The first symptoms of the Dilophospora disease, consisting of slight, silver green, blotches, appear on the uppermost leaf just as or even before its emergence from the sheath of the preceding leaf. The first, second, and in some cases even the third leaf, under natural conditions are as a rule never attacked by the fungus, and together with the base and underground portions of the plants remain free from the disease. Ranojewic (33, p. 398) also observed the disease only on the upper leaves.

The above facts throw an interesting light on the nature of this disease. The appearance of the disease first on the uppermost leaf shortly after or even before it has left the sheath of the preceding leaf and the freedom of disease of the lower leaves shows that the point from which the infection originates and spreads is the growing point of the plant itself. But how does the infection material reach the growing point of the plant without infecting the outer portions and the growing point of same?

All of the numerous infection experiments made with *Dilophospora alopecuri* alone gave absolutely negative results, in spite of the fact that infection experiments were made with wheat, spelt, and rye of various ages and of the same varieties on which the disease had been observed in nature; soil, seed, and wound infections at various temperatures and moistures of soil and air were also made, but the results were in all cases negative.

These results exclude the possibility that we have to do here with a fungus which attacks the plants in the way the smuts attack these plants or that this fungus behaves as some of the common plant pathogenic fungi.

Infection experiments in which wheat, spelt, and rye of different varieties were used and where the plants were infected simultaneously with *D. alopecuri* and *T. tritici* yielded in all cases positive and abundant Dilophospora infections even when comparatively large plants were infected. This showed that, regardless of whether the nematode attack on the plants makes them susceptible to the Dilophospora disease or not, the nematodes must certainly help in some way the fungus to reach the growing point of the plant.

From the life history of the nematode disease we know that from the soil the nematodes crawling and creeping between the leaf sheaths of the younger or older plants, reach their growing point. Under these circumstances it was natural to suppose that the nematodes carry the spores of Dilophospora to the growing point of the plants, where they subsequently attack the young leaves, though the way in which this is accomplished remained a question to be answered. Various experiments were made to establish this experimentally and if possible by means of the microscope.

For one of these experiments it was necessary to mix a water suspension of Dilophospora spores with a water suspension of nematodes. Immediately after the mixing of the two suspensions there appeared a heavy cottonlike precipitate in the container, while the liquid above the precipitate cleared up. A microscopic examination of the precipitate showed that it consisted of numerous nematodes, which were heavily loaded with Dilophospora spores, these having attached themselves to the nematodes by means of their bristles (Fig. 9), just as the seeds of some plants attach themselves and stick to our cloths, to the fleece of sheep or to the hair of other animals.

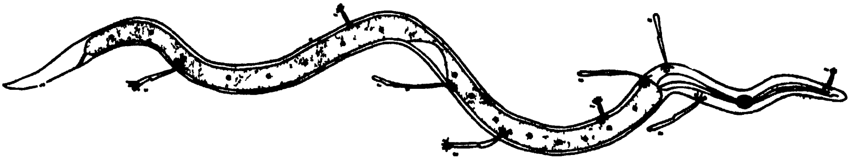

FIG. 9. Diagrammatical drawing of a larva of *Tylenchus tritici*. The larva carries a number of pycno–[1] and secondary[2] spores of *Dilophospora alopecuri*, which have attached themselves to it.

The spores are attached so firmly to the nematodes that they cannot easily free themselves from the spores, and even if they succeed sometimes in doing so they pick up again other spores in their subsequent movements.

The above fact strengthened considerably the supposition that the nematodes are the agents which bring the Dilophospora spores to the growing point of the plants, which also the nematodes must reach in order to assure their further propagation.

The further work on this problem, however, showed that upon examining under the microscope on the growing points of the infected plants, consisting of minute leaflets or the young head, there were to be seen in most cases numerous nematodes. A more careful examination of these nematodes showed that they carried in most cases more or less numerous spores of Dilophospora. These spores were not the pycnospores which originally were applied together with the nematodes, but the secondary spores of the fungus, as observed in young cultures of the same. This condition can be seen even before the appearance of the Dilophospora symptoms. This shows that the original pycnospores of the fungus with which the nematodes must have entered the plant have meanwhile germinated and have formed many secondary spores, just as they do in artificial cultures. The number of secondary spores found on the growing points of infected plants is often very large, so that they can be easily located upon microscopic examination of the vegetative point in water. With the advance of the fungus disease the nematodes die or leave the plants; this is especially true of plants severely attacked by the Dilophospora disease.

Examination of the otherwise perfectly healthy and normal growing points or rudimentary heads of a large number of plants infected naturally and artificially with Dilophospora showed the presence of nematodes which in all cases carried more or less numerous secondary spores of the fungus. In the examination of the plants for nematodes and spores all precautions were taken to avoid external contamination of the growing points during the opening and subsequent handling of them.

The finding of the spore carrying nematodes at the growing points of the young plants, diseased with Dilophospora and those infected but not yet diseased, confirmed the supposition that the fungus spores reach the growing point of the plants by means of the nematodes. But is this sufficient for the successful establishment and development of the fungus on the thus infected plants? If the nematodes do nothing more than merely deposit the spores of the fungus upon the tender and succulent growing point of the plants, it should be possible to infect plants with Dilophospora by depositing the spores of this fungus upon the growing points of the plants in a mechanical way.

About fifty wheat and rye plants in various stages of development were infected with Dilophospora by splitting the plants slightly just at or above the growing point and placing a great number of spores in the central cavity of the plants. The wounds of the plants were then closed and tied up. Other plants were infected by injecting in their central cavity a heavy suspension of Dilophospora spores. Still other plants were infected and reinfected in the above ways every other week, beginning as soon as they had their third leaf and continuing until they headed out. In all cases the plants failed to develop the disease.

Must it be concluded from this that Dilophospora alone can not infect even when present on the growing point of the plants? An absolutely positive answer to this question can not be given at this moment. The fact however that the Dilophospora diseased plants will recover fully from the disease if not already vitally injured, and produce perfectly healthy and normal leaves above the diseased ones as soon as the nematodes in them die, leave them, or have formed galls, indicates that the relation between the two pathogenes is a more intimate one.

From the life history of the nematodes we know that they are pushed continuously upwards by the growing point of the plants, but we know from the morphologic development of the cereal plants that what has been a growing point a few days or even hours ago is now a leaf and that, if the nematodes did allow the growing leaves to push them upwards, they would soon come in this way outside of the plant. This however does not happen, because the nematodes desert continuously the newly differentiated leaves and move constantly downwards to the vegetative point, taking along also the Dilophospora spores. When the nematodes die or leave the plants, as pointed out above, it is natural that the Dilophospora spores which may have been deposited by the nematodes on the vegetative point, are thrown out with the differentiation and growing out of the new leaves. Thus the growing point of the plant and its whole central cavity are freed from the spores of the fungus. This explains why the leaves formed after the nematodes leave the plants, are free from the Dilophospora attack. The last fact can be interpreted also in the following way: The nematodes not only bring the Dilophospora spores into the central cavity of the plant and carry them subsequently back to the growing point of the plant from its growing out leaves, but the nematodes sucking or otherwise injuring or affecting the plants cells of the tender and minute leaves open the way for the penetration of the fungus into the cells and tissues of the young leaves and afterwards of the young head. This supposition seems to be supported also by the fact that, so far at least, all wound infections with Dilophospora have given negative results. That in the case of the wound infection the infection material has actually been placed on the growing point or the young head of the plants is shown by the fact, that a large number of the heads of such plants were deformed at the time they appeared from the leaf sheaths and some of them bore even the infection material (fractions of pycnidia and numerous spores) which had been placed upon them while still very small and lying deep in the sheaths. But none of the deformed and artificially infected heads showed any symptoms of the disease and were free from any fungus attack.

As is well known, the nematodes sometimes die or desert the weak, sickly or wilting plants; this they do also with the heavily Dilophospora infected plants. As soon as they leave the plants these recover, send out new leaves and even new tillers, if their primary stems have been killed by the fungus, which are free from the disease and may even form normal heads. This also happens when the nematodes bore into the young heads and form galls.

Though the boring of the nematodes into the floral parts of the young heads lessens the danger of infection for the uppermost leaves, it must be the initial cause for the establishment of the fungus at last also on the heads. The top of the heads being the first to be formed is most commonly attacked by Dilophospora. The heads of plants severely attacked by the fungus are destroyed at an early date by the downward advancing mycelial growth from the bound together upper leaves.

As a rule the heads of Dilophospora infected plants, if ever formed, appear from the upper leaf sheath with distinct and prominent symptoms of the disease. Those which come from the sheaths free from the disease remain healthy.

The infected heads, as already stated, are not wholly destroyed by the fungus. In the apparently healthy portions of such heads are formed nematode galls and even normal kernels. The partially infected heads are, from the standpoint of the Dilophospora disease, of great importance because the nematode galls formed on them are often infected and overgrown by the fungus. Such galls thus became the carriers not only of the nematodes but also of Dilophospora. With the carrying of these galls to the fields planted or to be planted with cereals they give rise again to both diseases and thus complete the life cycle of both pathogenes.

The above fact explains why in the writer's infection experiments the plants to which were added no Dilophospora but only nematodes developed both diseases in all cases. The nematodes used in these infection experiments originated from a field that was attacked both by the nematode and Dilophospora diseases. At first it was thought that the soil must have been naturally infected with Dilophospora. The same experiments repeated on sterilized soil gave similar results. Further infection experiments in which only sterilized soil and only the nematodes from large, externally uninjured, black and shining galls, that were not overgrown by fungi, were used, yielded a smaller percentage of Dilophospora sick plants, than the check plants where the nematodes of average galls were used. These experiments show that in the above case at least some of the galls were infected by Dilophospora and that this is sufficient for the subsequent development of the Dilophospora disease.

For the simultaneous occurrence of the two diseases on the same plants in nature it is, however, not necessary that the nematode galls be infected with the fungus. In moist weather the spores of the ripe pycnidia leave the latter in the form of a thread or tendril, so that soon there appears over the infected area a very thick layer of spores. These spores germinate forming secondary spores so that the layer of spores becomes still thicker. It is highly probable that the nematodes after leaving the galls and while creeping around over the stubble in the fields must naturally come in contact with such spores and take along some of them on their way to the plants.

A very slight contamination of the nematodes with spores of Dilophospora is not only sufficient but is a prerequisite for the appearance of the Dilophospora disease. Infection experiments where, to the nematode suspensions were added heavy spore suspensions, yielded in most cases absolutely disease free plants, *i. e.*, both diseases failed to develop on the infected plants. A heavy or even moderate contamination of the nematodes with spores is absolutely fatal for both diseases. Only such infections, where to the nematode suspensions were added very slight spore suspensions, yielded results. Severest infections of Dilophospora were obtained where no Dilophospora spores were added to the nematode suspensions. In such cases 100 per cent of all infected plants developed symptoms of both diseases and a microscopic examination of the vegetative points of the plants showed the presence of numerous nematodes, always loaded with secondary Dilophospora spores.

All galls which the writer had at his disposition, originating from fields where both diseases appeared simultaneously, were evidently infected by Dilophospora, because when used as infection material they produced in all cases both diseases. A microscopic and cultural study of the galls and the fungi present on them in no case established with certainty the presence of Dilophospora spores or mycelia on or in them. The great size of the galls and the many thousands of nematodes in them make it impossible to see under the microscope the whole content of a gall; on the other hand the very slow growth of this fungus makes it impossible to demonstrate its presence by plating or diluting the contents of the galls in agar, since the numerous other fungi which are usually present on the galls overgrow the plates long before the germination of the Dilophospora spores.

All efforts to secure Dilophospora free galls with viable nematodes were unsuccessful, so that important questions in connection with this problem must wait their solution until such material can be obtained.

CONTROL OF THE DISEASE

The nature of the disease in this case itself shows the way for controlling it: Preventing the nematode disease means also controlling the Dilophospora disease; which, as already pointed out, occurs only on plants infested by the nematodes.

Various chemicals and the hot water treatment have been recommended to control the one or the other of these two diseases, but in most cases they are not easy to apply or fail to give satisfactory results, while as regards the Dilophospora disease they have never been tried.

For controlling the nematodes the following measures have been used successfully and can be recommended.

Clean Seed

It is generally known that the main source of nematode and therefore of Dilophospora infection is the seed. For preventing both diseases it is therefore absolutely necessary to use clean seed, which is free from nematode galls and originating from fields where the diseases have not been present. Where this is not possible the seed should be freed from the nematode galls before sowing. This can easily be accomplished by the so-called sedimentation or salt-brine method. Byars (3, 4) used with success a 20 per cent common salt solution.

The seed is treated in the following way: First make up a 20 per cent salt solution by dissolving 20 Kgr. salt in 100 liters of water. Then pour the seed gradually into this solution, stirring vigorously at the same time. The sound wheat or rye kernels will sink while the nematode galls, light kernels, and trash, because of their lower specific gravity, will float. The floating galls and other contaminations are then skimmed off and boiled or burned to kill the nematodes. When upon further stirring of the liquid no more galls come to the surface, drain away the salt solution, which may be used repeatedly, and rinse at once in water. The treated grain is spread on the floor or a canvas to dry. The drying of the seed should be as rapid as possible, otherwise it may begin to germinate. The rinsing of the grain in water should also be done promptly, otherwise the viability of the grain may be injured. On account of this it is more advisable to use the same proportions of potassium chloride, instead of common salt, because when applied in the same way it does not injure the seed and, besides this, it can be used as a fertilizer after the treatment.

Spring wheat and rye treated both with salt and potassium chloride solution gave the germination results shown in table 2.

That a separation of the galls from the seed will eliminate both diseases was shown very conclusively in one of the writer's experiments, where three plots of 20 M² were sown with hand picked wheat, rye, and hulled spelt. All of these three kinds of seed were taken from a heavily infected mixture of wheat, spelt and rye. The three plots planted with hand picked seed, *i. e.*, with gall free seed, were perfectly free from both diseases. Whereas a fourth plot planted with the original mixture, developed both diseases in great abundance.

Sanitation

Besides with the seed, the nematode galls and nematodes may reach the fields with the straw or stable manure, or they may be brought over from

TABLE 2—*Germination of rye and spring wheat treated with a 20 per cent solution of NaCl and KCl. The treated seed was left at room temperature for 15 hours to dry and was planted in sand. Counts taken 15 days after planting.*

Number	Kind of seed	Treatment	Aftertreatment	Number of kernels	
				treated	germinated
1	Rye	no	no	400	254
2	"	H_2O	no	400	265
3	"	NaCl	no	400	188
4	"	NaCl	H_2O	400	226
5	"	KCl	no	400	214
6	"	KCl	H_2O	400	249
7	Spring wheat	no	no	400	301
8	" "	H_2O	no	400	292
9	" "	NaCl	no	400	265
10	" "	NaCl	H_2O	400	284
11	" "	KCl	no	400	274
12	" "	KCl	H_2O	400	328

infested fields to an uninfected area by means of the farm implements or surface water. Therefore no infested grain or straw should be used as animal food or the manure of animals so fed should never be applied to fields that are to be planted with wheat, rye or spelt. No surface water should be allowed to pass from infested to uninfested areas. A deep ploughing under of the stubble just after harvesting lessens the spread of the disease to nearby fields. Since most of the lighter galls are blown away in the straw during the threshing, it is highly desirable to burn up the straw of infested fields on the very place of threshing and as soon as this is finished.

Crop Rotation

Some of the nematodes present in the soil may fail to reach the plants and thus remain in the soil for a longer time, and a number of the newly formed galls must naturally remain in the fields after the harvesting of the crop. For this reason no susceptible crops should be grown on the same field for at least one year. Lengthening this period to 2 or 3 years will eliminate completely the disease. The larvae of *Tylenchus tritici*, contrary to those of most nematodes, can not remain alive for a long time in the moist soil, as Johnson and Leukel's (18) work shows, and die within a year, but it is probable that under certain conditions some of them may remain alive for a longer period of time.

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OBSERVATIONS ON BARK DISEASES OF CITRUS TREES IN SICILY

HOWARD S. FAWCETT

When passing through Italy and Sicily in May, 1923, the author had an opportunity to compare some of the bark diseases there seen with some of those he had previously been studying in California and Florida. Lesions which appeared to belong to the following bark diseases as now known in California were noted: Pythiacystis gummosis, decorticosis (shell bark), citrus blast and black pit on lemon trees, and psorosis on orange trees.

Pythiacystis Gummosis. Lesions with the characteristics of those produced by *Pythiacystis citrophthora* Sm. & Sm. in California were seen on orange and lemon bark in a few cases where trees were budded low. As has been pointed out,[1] however, lesions produced by this fungus cannot be told with certainty superficially from those produced by *Phytophthora terrestris* Sherb. The characteristic invaded patches of bark, killed to the wood, mineral brown at the cambium, surrounded by a yellow ochre gummous zone, the exudation of gum on the surface and the characteristic odor were all typical of either *Pythiacystis citrophthora* or *Phytophthora terrestris.* Most of the old lemon trees in Sicily at the present time are budded very high (sometimes 4 to 5 ft.) on sour orange (*Citrus Aurantium* L.) stocks, the bark of which stock is nearly immune to this disease. For this reason the lemon orchards are for the most part now free from this type of gummosis, although from 1863 to 1870 nearly all the trees (at that time mostly on other stocks) were killed by it.[2]

Decorticosis (shell bark). This disease was also observed frequently on old lemon tree trunks in a form quite similar to that occurring in California.[3] In California this disease is now thought to be due to a species of Phomopsis, *P. californica.*[4]

Many old lemon trunks showed the same cracking and shelling off of the outer bark in elongated strips as that seen here. The outer bark is killed and later in drying out it shrinks, warps and partly loosens from the inner portion which remains alive and builds up a new outer bark.

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[2] Savastano. Pathologia arborea applicata, p. 127–141. Napoli. 1910.

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Citrus blast and black pit. These two manifestations of *Pseudomonas citriputeale,* citrus blast[5] and black pit,[6] were frequently seen both in Sicily and on the peninsula of Italy near Naples. On meeting Prof. Savastano at the Agricultural Experiment Station in Acireale, Sicily, it was found that he had already recognized the identity of the first one, citrus blast. The black pit was found on the fruit as a common accompaniment of the other. Both lesions were typical of those occurring in California. Recently Savastano[7] has mentioned both forms and thinks that the same organism is responsible for certain lesions on the trunk near the ground. Savastano reports that the citrus blast form of the disease has been in Sicily for a long time. Until recently it was not known by us[8] to occur outside of California.

Psorosis. This disease which is common to orange trees in both California and Florida, the cause of which is unknown,[9] was also seen on old orange tree trunks in a few places in Sicily and Italy. The symptoms all appeared to be the same as those for trees affected here. There was the same formation of irregular scales by the drying out and loosening of the outer bark, the formation of droplets of gum on the surface and an irregular semi-hypertrophied new tissue forming under the old loosened outer bark.

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PHYTOPATHOLOGICAL NOTES

Bibliography der Pflanzenschutzliterature.—The attention of American plant pathologists was called to this publication by Spaulding in 1921 (Phytopath. 11 : 377). Although issued as an exchange journal Dr. Morstatt, the editor, reports few requests for it from American institutions. It has been issued in one volume annually since 1921. It appears to give a rather complete bibliography of the world's literature on the diseases and pests of plants for the year. It is not an abstract journal but the articles listed are fully and carefully cited, including full title in the original language. The papers cited are arranged according to a very convenient classification, being listed alphabetically by authors under each sub-division. This is a publication which every American plant pathologist and economic entomologist should have at hand as it presents in convenient form the year's output of literature in the field of plant protection, especially that of our European colleagues. This bibliography is issued by the Biologische Reichsanstalt für Landund Forstwirtschaft, Berlin-Dahlem. If your institution is not receiving this through regular exchange, you should see to it that it does so, for you are missing something worth while. The 1923 issue contains approximately 4,300 references.—H H. WHETZEL.

The Board of Agriculture for Scotland has just established a department of "Plant Pathology Investigation." Mrs. N. L. Alcock is the mycologist in charge. The new laboratory may be addressed at the Royal Botanic Garden, Inverleith Row, Edinburgh. Since this is the first time that an institution has been officially assigned to work upon mycology and plant diseases, the authorities will be very grateful for reprints and other literature on these subjects for their library.—PERLEY SPAULDING.

ABSTRACTS OF PAPERS PRESENTED AT THE SIXTEENTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, WASHINGTON, D. C., DECEMBER 30, 1924 TO JANUARY 1, 1925

Use of agar blocks for photographing living spores. C. D. SHERBAKOFF.

To keep living spores stationary and in one plane which is necessary for the best photographic reproduction a method was devised in which, for a study or photographing, the water-drop method was substituted by agar-block method. The blocks are cut out of agar sheets made by pouring clear agar between glass slides separated from each other by halves of very thin glass slides. The spore material is placed on a very clear cover glass into a small droplet of water and evenly smeared over it; the spores are allowed to become nearly dry, until the surface begins to look like that of a ground glass, and then turned over upon the agar block on the glass side. With this improvement long exposures can be made and a field selected where a great number of spores have arranged themselves in a desirable way.

Masuring water flow interference in diseased stems. I. E. MELHUS, J. H. MUNCIE and WM. T. H. HO.

A piece of apparatus has been devised for measuring the rate of flow in diseased and normal plant stem tissues. This apparatus consists essentially of a filter pump which exhausts the air from the system, a mercury column, a side delivery burette and a separatory funnel. These are so connected as to pull water or stains through stem tissues under at least one atmosphere of pressure. This apparatus, called a fluometer, makes it possible to measure the flow interference caused by *Bacterium tumefaciens, Fusarium conglutinans* and certain other gall and vascular parasites. Tests on 100 apple trees-galled with *Bacterium tumefaciens* averaged 30 per cent reduced water flow. Cabbage plants infected with *Fusarium conglutinans* showed a partial or complete stoppage, depending upon the extent of the vascular invasion by the parasite. The normal cabbage stems averaged 16.02 c.c. flow in five minutes.

Cacao canker in Java. CARL HARTLEY.

Cacao canker is a red rot of the outer bark, which reaches the cambium only on the most susceptible trees. Its most rapid spread is tangential in the outer, and longitudinal in the inner, bark. *Phytophthora faberi* (?), which causes the disease, is difficult to isolate directly except from rapidly spreading lesions. It can be more easily obtained by using marginal canker tissue as crude inoculum in controlled wound inoculations on immature cacao pods; from the successfully inoculated pods the fungus is easily isolated. By removing the bark from cambium-killing lesions, sporulation can be induced on the underlying wood. In most countries the Phytophthora infections are reported more numerous in the pods than in the bark; in Java, on the other hand, cankers are often more numerous than rotten pods. Phytophthoras obtained from cacao differ decidedly from each other both morphologically and physiologically. The promiscuous cutting out of lesions appears to do more harm than good in Java, and the superficial shaving of lesions recommended by a number of writers is of very dubious value. The great variation in resistance of cacao to canker appears to be the key to the decrease of the damage from this disease.

The influence of temperature and of previous infection on the development of crowngall. A. J. RIKER.

The effect of temperature on the development of crowngall on aerial parts of tomato stems was tested in chambers where the air temperatures and moistures were regulated. Well developed galls were secured between 14° and 28° C. At 28–30° C. only poorly developed galls were produced and above 30° C. no galls appeared. The host plants made fair growth at these temperatures. At 8–10° C. the inoculated plants made practically no growth and produced no galls in a month.

The temperature relation of the causal organism was studied in nutrient dextrose agar, in modified Colley's medium, and in tomato broth. Although growth appeared over a much wider range, vegetative development was active only over the range of temperatures approximating that where galls were secured, *i.e.*, 14° to 30° C.

As others have reported, previous infection did not appear to influence crowngall development. A search for agglutinins or precipitins in plants which bore large galls, or in the galls proper, gave negative results. However, after the crowngall organism had been injected into a rabbit a serum was produced which possessed agglutinating properties in dilutions of 1 to 3,000.

Mottle-necrosis of sweet potatoes. L. L. HARTER.

In a brief article published in 1923 attention was called to a field disease of the enlarged roots of sweet potatoes called "mottle-necrosis," which was more or less widely distributed throughout certain sections of the country. It was pointed out that although a large number of attempts had been made to isolate an organism, plantings of necrotic tissue in agar plates either remained sterile or gave an occasional growth of Fusarium or bacteria. Since publication of the above article the disease has been located in new regions, indicating that it is probably spreading. By applying a special type of technique in the further study of this disease, the writer has been able to isolate a species of Pythium from the dead tissue in almost every case. It is believed that in view of the almost constant association of Pythium with the disease and of the results of some preliminary inoculations in the laboratory that Pythium is probably the causal organism.

Studies on the cytology of sugar cane mosaic. MEL T. COOK.

The white areas of infected leaves are thinner than the green areas or than normal leaves. Intercellular plasmodial bodies are frequently observed in the white areas. The nuclei in the white areas are more conspicuous than in the green areas or normal leaves because of protoplasmic masses. These masses may be spherical, irregular or elongated. The nucleoli are more numerous in the diseased than in the healthy cells and the chloroplasts are reduced in both size and number. Plasmodial bodies are found in all tissues of the stem except the very outer ones. They are always most conspicuous in actively growing parts, such as leaves that have just unfolded and in the parts of the stem just back of the growing point. They are not easily found in the older parts of the leaves or stems, or in old cankered canes. A disintegration of the walls of the internal cells of the canes is of very frequent occurrence, thus leaving protoplasmic masses, but the writer has been unable to associate plasmodial bodies with this condition. The presence of multi-nuclei and granular masses in the cells is not restricted to mosaic canes. The photosynthetic activity of the mosaic canes is greatly reduced.

Selecting for resistance to the sugar cane mosaic. C. W. EDGERTON.

By selecting plants showing very little effect of the mosaic in fields that have been 100 per cent infected for a number of years, strains have been obtained which show a very high resistance to the disease. These strains have been carried through three years without losing their resistant characteristics. With a vegetatively propagated plant, this means either that bud variations are occurring or else that certain plants are acquiring an immunity to the disease.

Geographical distribution of the milkweed flagellate, Herpetomonas elmassiani (Migone). Its non-pathogenicity in Maryland. FRANCIS O. HOLMES.

The flagellate inhabiting the latex of the milkweed *Asclepias syrica* L. was most widespread during September and early October. At that time a search was made for the northern limit of its range along the Atlantic coast.

In Maryland the flagellates were as common as they were last year. They could be traced north as far as the New Jersey bank of the Hudson River. But five hundred milkweeds collected in Massachusetts, and an equal number collected in Yonkers, N. Y., were negative. On the coast the normal limit for *Herpetomonas elmassiani* (Migone) in milkweed seems to be northern New Jersey.

In Maryland heavily infected and uninfected plants growing side by side were indistinguishable in total height, in color, and in the number of seed pods produced. Though they were under observation from June to September it was not possible to predict which plants were positive and which negative without a microscopical examination of the latex for organisms. Apparently the plants were capable of supplying abundant food to the flagellates without limiting their own growth.

Effect of intermittent temperatures on potato mosaic. C. M. TOMPKINS.

It has already been shown by Johnson that constant exposure to temperatures above 24° C. masks potato mosaic and that lower temperatures favor mosaic symptoms. Experiments in constant air control chambers now indicate that relatively short exposures to the higher temperatures are sufficient to completely mask mosaic. Mosaic plants placed for 9 hours at temperatures above 24° C. but remaining at temperatures favorable for mosaic for 15 hours recovered in 7 days. Mosaic plants exposed for only 2 hours at 28° C. but at favorable temperatures for mosaic for 22 hours practically recovered in 28 days. At 40° C., however, a 2-hour exposure gave recovery in 7 days, and a 6-hour exposure enabled recovery to occur in 4 days.

At temperatures above 24° C., new leaves produced are not mottled or crinkled but are a uniform dark green, lacking all symptoms characteristic of mosaic. Masking often seems to be associated with stimulated chlorophyl production. The lower or older leaves with distinct symptoms may or may not lose their mottling and crinkling completely, depending upon the age of the leaves, upon the duration of exposure, and upon the temperature to which exposed at or above 24° C. In plants where mosaic was masked by high temperature, characteristic symptoms were again induced by exposing the plants to favorable low temperatures.

A virus from potato transmissible to tobacco. JAMES JOHNSON.

Symptoms of disease can be obtained on tobacco plants inoculated with extract from mosaic potato foliage. These symptoms are distinctly different from tobacco mosaic and are generally characterized by faint mottling or irregular necrotic areas. Whether or not this disease is potato mosaic on tobacco is not easily determined. Other potato degeneration diseases and, as far as can be determined, healthy potato foliage usually yields the same symptoms as mosaic potato foliage.

A combination of potato mosaic and tobacco mosaic virus on tobacco produces another symptom, different from either one alone. Leaf spots and blotches, sometimes killing entire leaves, normally occur, whereas such symptoms do not occur with tobacco mosaic alone in the controls. Similar results were secured on tomatoes.

The causal agent of both the single and the combination symptoms on tobacco can be transmitted repeatedly from tobacco to tobacco.

Either potatoes free from this virus are rare or else extract from healthy potato foliage may cause a physiological disturbance of tobacco and tomato plants, which is of an infectious nature. Assuming the latter, it suggests some interesting possibilities as to the origin of virus diseases.

The Fusarium Conference. C. D. SHERBAKOFF.

The subject in general will be covered also in a paper prepared by the members of the conference. The conference met in Madison, Wisconsin, early in June, 1924, and continued for two months. The following members regularly attended it: Dr. H. W. Wollenweber, from Germany; Dr. Otto Reinking, of the United Fruit Company, and Dr. Helen Johann and Mrs. Alice Bailey, of the Bureau of Plant Industry, and myself temporarily employed for the purpose by the Bureau of Plant Industry in cooperation with the Tennessee Experiment Station. Dr. Wolllenweber was employed by the United Fruit Company to study Reinking's Fusaria, and the rest of us to increase our knowldege on the subject. Besides the identification of Reinking's Fusaria by Dr. Wollenweber, the conference prepared a paper on "Fundamentals for taxonomic studies of Fusarium," revised the names in "Fusaria of Potatoes," defined Lisea section and subsection Ferruginosum, made a revision of section Elegans, and examined certain special sets of cultures of Fusaria. The significance of the conference lies in its international character and in the earnest cooperation of a commercial enterprise and several public institutions in a purely scientific subject.

Leaf-spot of maize, a disease distinct from leaf-blight. CHARLES DRECHSLER.

Longitudinally elongated buff lesions on maize leaves from Florida and the Philippines, typically much smaller and more numerous than those of leaf blight, and often delimited by the veins of the host, have been found due to a species of Helminthosporium distinct from *H. turcicum* Passerini, differing from the latter especially in its strongly curved, elliptical conidia being more narrow, more abundantly septate, only slightly tapering from the middle, and provided at the base with an internal scar rather than a protruding modification. Discrete, subglobose, distinctly beaked perithecia developing subcylindrical short-stipitate asci with typically 4 fuliginous, filamentous, 5-to-9 septate spores disposed in a close quadruple heterostrophic spiral, each spore usually making 4 turns, are abundantly produced. Under experimental conditions the fungus is violently pathogenic to maize but causes only incipient lesions on rice and sugar cane. The morphological difference between the ascigerous stage and Pyrenophora or Pleospora supports the view that the species of Helminthosporium with straight subcylindrical conidia germinating laterally from end and intermediate segments, constitute a natural group distinct from the large group of species, which like the leaf-spot parasite, produce curved elliptical conidia germinating by two polar germ tubes.

Tabulation of the genera Phoma and Macrophoma. P. A. YOUNG.

This paper consists of a tabulation of the species of Phoma and Macrophoma given in Saccardo's "Sylloge Fungorum" and most of the more recently described species. The arbitrary difference between these genera has not been well observed. They intergrade with Phyllosticta, Dendrophoma and Septoria. The species are tabulated in the order of their minimum spore lengths and further arranged in the order of maximum spore lengths. The tabulation contains a reference to a description of each species, sizes of pycnidia, ranges of spore lengths and widths, family numbers and host genera. The columns of family numbers and generic names serve as a host index. Phoma is probably the largest genus of plants. The tabulation contains 1,563 species of Phoma and 314 species of Macrophoma; 148 species being omitted because their descriptions lacked spore lengths. The existence of 612 species with minimum spore lengths between 3 and 5 microns points out one of the repelling difficulties of classifying species of Phoma.

Cytological studies of crowngall tissue. A. J. RIKER.

Comparisons of cells which developed in tomato plants after infection by crowngall bacteria, and after wounds, have shown a progressive reduction in the sizes of the cells and nuclei, and in the nucleo-cytoplasmic ratio to a minimum from which there was a slight return. The minimum was reached more quickly by the wound tissue than by the gall tissue. Several types of inclusions were observed in different kinds of crowngall cells. In tomato galls no clear evidence was found that nuclear division occurs by amitosis or that more than one nucleus appears in an individual cell. As others have observed, secondary thickenings in the walls first appeared among the smaller and more active cells. There seems to be a close similarity between the cells in crowngall and wound tissue.

Sweet potato varieties that produce well and are resistant to stem rot on sassafras sands. R. F. POOLE.

By testing a large number of sweet potato varieties and strains in New Jersey on sassafras sands infested with *Fusarium batatatis* and *F. hyperoxysporium,* three varieties were found to be resistant to the stem rot disease and to yield well on most of the infected soils where the native Jersey varieties were grown at a loss due to heavy kill by the disease. The results obtained with the Red Brazil, the White Yam and the Triumph varieties have made a very favorable impression on many growers. Seed, especially of the first two varieties, has already become widely distributed in this state where herebefore only the Jersey varieties were grown for commercial purposes.

The relation of soil moisture to the pox disease of sweet potatoes. R. F. POOLE.

Feed and edible roots of the Vineless Yellow Jersey variety of sweet potatoes were severely infected by *Cystospora batatae,* the causal organism of the pox disease, when grown in infected soil maintained at 4 and 6 per cent moisture. At 8 and 10 per cent moisture the edible roots were not attacked, but the feed roots showed a large number of poxed spots. At 12, 14 and 16 per cent the infection was very slight, while the maximum productions of both raw potatoes and total dry weights were obtained. At 18 per cent (soil saturation) there was no infection. These results explain the data obtained from field observations, which showed that the pox disease was so severe in dry seasons that it often resulted in a failure of the crop, while on the same soil the disease was much less severe and the crop a success in wet seasons.

Second progress report of black rot (Pseudomonas campestris) investigations on Long Island: seed infection and seasonal development. E. E. CLAYTON.

In greenhouse experiments cauliflower seed plants, spray inoculated, developed pod lesions in seven days, while the incubation period for succulent young plants was 13 to 15 days. Inoculations of seed pods of cabbage and Brussels sprouts were also successful. The organism remained alive for three years in naturally infected seed.

Infected seed sprouted normally, the disease first appearing as a cotyledonary lesion from which bacteria spread into the main stem. Progress up and down the stem was slow and infected plants appeared normal at time of transplanting. The first field symptom was the wilting of one or more lower leaves. This was followed by secondary spread which was most intensive in the immediate neighborhood of primary infected plants.

With cauliflower the major portion of secondary infection took place directly thru the uninjured lower leaf surface. Following favorable weather individual leaves showed 100 or more lesions. Such infections occurred also commonly on Brussels sprouts and occasionally on cabbage.

Stringent protection against all insects did not check the spread of the disease. The principal agencies of dissemination were rain and plant contacts incident to cultivation.

Second progress report on seed treatment for black leg (Phoma lingam) and black rot (Pseudomonas campestris) of cruciferous crops. E. E. CLAYTON.

In 1924 seven lots of infected seed (three infected with black rot and four with black leg) of cauliflower, cabbage and Brussels sprouts was used. Each treatment was represented by separate seed bed and field plats.

Hot water treatments of 14, 16, 18, 20, 25 and 30 minutes at 50° C. gave good, but not complete, control of black leg and, with one exception, complete control of black rot.

Seed treatments with Uspulun 0.25 per cent for 0.5, 1 and 2 hours; Semesan 0.25 per cent for 0.5, 1, 2 and 4 hours; $HgCl_2$ 0.1 per cent for 0.5 hours; and HgI_2 0.05 per cent plus KI 0.1 per cent for 1, 2 and 4 hours all gave unsatisfactory control of both diseases.

In experiments with one lot of seed infected with black rot, Germisan 0.25 per cent for 0.5 and 2 hours, and $Hg(CN)_2$ 0.1 per cent for 1 hour gave complete control but the latter much reduced germination.

Dust, Dupont No. 13 (an organic compound of mercury) gave excellent control of black leg but was completely ineffective against black rot.

Alternaria Solani as a cause of tuber rot in potatoes. DONALD FOLSOM and REINER BONDE.

The fungus was isolated from lesions in tubers taken from commercial storage in different years and places. The lesions were distinctive, being darker than the healthy skin, somewhat sunken, and surrounded by a slightly raised border which was circular or irregular in outline. A typical lesion is shallow, rarely exceeds a centimeter in diameter, and is easily removed apparently because of being limited by a new cork layer. With the isolated cultures positive results were obtained from the inoculation of young tubers and of foliage of greenhouse plants. The fungus was reisolated from the tubers and used for successful reinoculations. Immature tubers packed in diseased foliage secured from the field became badly infected and all attempts at isolation from these tubers gave pure cultures of the fungus. It is thought that under Maine conditions the chief loss involved with this type of rot is due to secondary infection. In two lots, one culture in about every ten was incapable of producing the usual reddening of potato agar, though the spores and infectiousness were normal, indicating the existence here of a saprophytic rather than parasitic difference between biological strains.

Drop of Chinese cabbage and common cabbage. W. H. DAVIS.

In the late summer and autumn of 1923, sclerotia were observed on diseased Chinese cabbage (Petsai, Chokurei, Wong Bach, Kinshiu, Chosen) and our common cultivated cabbage (Danish Baldhead) growing in the gardens of the Massachusetts Agricultural College at Amherst. The writer has not observed in literature any previous reference to this disease on Chinese cabbage.

Mycelium and apothecia were cultured from the sclerotia and measurements of their parts showed the fungus to be morphologically identical with *Sclerotinia sclerotiorum* (Lib.) Massee. (*Sclcrotinia libertiana* Fckl.) which causes lettuce drop.

While a *Botrytis sp.* was isolated from and grew abundantly on dead portions of Chinese cabbage, it was not associated with the life cycle of the Sclerotinia.

Inoculations within the host species and reciprocal inoculations of Chinese cabbage (*Brassica Pe-Tsai* Bailey), cultivated cabbage (*Brassica oleracea* var. *capitata* L.) and lettuce (*Lactuca sativa* L.) gave positive results.

Teratological forms were observed in which the margins of the apothecia formed secondary apothecia by proliferation.

Dusting celery seedbeds to control blights. A. G. NEWHALL.

Both the bacterial blight (*Pseudomonas apii*) and late blight (*Septoria apii*) have frequently been found on plants in the seedbeds. It has been shown that dusting the seedlings from 2 to 4 times with 20–80 copper-lime dust at about weekly intervals before transplanting them to the field has greatly reduced subsequent development of blights. From 4 to 20 times as much blight has been found later in the season on plants from the untreated portions of the seedbeds. Increases in yields of from 25 to 100 crates per acre have beeen obtained by dusting the seedbed or by locating the seedbed on new muck to insure blight free plants for transplanting.

The significance of the 1924 outbreak of western yellow tomato blight in the United States. MICHAEL SHAPOVALOV.

Western yellow blight of tomatoes was exceptionally severe in the summer of 1924 in several widely separated regions of the country. It destroyed nearly 100 per cent of the crop in one locality in California and about 30 per cent of the total commercial crop of the state of Utah. Moreover, it was during that season that it was for the first time reported east of the Rocky Mountains where heretofore its occurrence has not been known. This unusual outbreak of the disease was due to a peculiar combination of climatic factors which tended to produce a high rate of evaporation. In those localities where the amount of blight was greater than ordinary, the rate of evaporation was decidedly higher than the normal; and vice versa, in the localities where the disease appeared in the usual percentage, the rate of evaporation was about the same as usual.

The blossom-end rot of pepper. B. B. HIGGINS.

In a previous publication (Ga. Exp. Station Bul. 141: 61–62) a disease of pepper, *Capsicum annuum* L., was described and tentatively ascribed to physiological causes, principally variations in moisture supply. This conclusion was based upon field observations and the fact that no organism was found in the tissue of newly formed spots.

During the last two years blossom-end rot has been repeatedly produced experimentally by manipulation of the water supply. In general terms, lesions develop near the blossom end of young, rapidly growing, pepper fruits when transpiration is high and soil moisture not sufficient to supply the needs of the plant. A histological study of young spots has shown that they are initiated by a collapse of large cells about the ends of the small veinlets which are abundant near the blossom end of the fruits.

The variety as a unit in studies of disease resistance. L. J. STADLER.

Determinations of varietal resistance to disease imply the assumption that different stocks of an agronomic variety are practically equal in resistance to a specific disease. Percentages of infection were determined in the seasons of 1922–1923 and 1923–1924 in 50 stocks of Fulcaster wheat inoculated with *Tilletia laevis* and in 57 stocks of Kherson oats inoculated with *Ustilago laevis* and with *U. avenae.* The stocks of each variety were taxonomically pure and identical, and were obtained from seedsmen and experiment stations in different parts of the United States. In all cases marked differences in the percentage of infection between different stocks of the same variety were found. Conclusions regarding varietal susceptibility may often be in error, because the stocks tested do not represent the varieties in which they belong. The results of a disease resistance test may properly be applied only to the strains actually tested.

Inheritance of disease resistance in wheat and oats. E. F. GAINES.

Recent literature is reviewed showing that commercial varieties of farm crops vary greatly in resistance to their major diseases. When resistant or immune varieties are crossed with susceptible ones, resistant and susceptible segregates are obtained in the F_2 generation which breed true in subsequent generations.

In testing more than five hundred varieties and selections of wheat in respect to resistance to bunt, during the past ten years, the author finds variations from immunity to complete susceptibility. Crossing varieties and testing the progeny in subsequent generations has shown that resistant wheats may carry different factors for resistance which are cumulative in effect. From one such cross an immune variety has been produced which has sufficient merit for commercial production.

In testing two hundred and eight varieties of oats for resistance to covered smut, twenty-three were found to be immune. When the immune Red Rustproof was crossed with four varieties of varying susceptibility, a large percentage of the F_3 families were immune. Hull-less segregates occurred which could not be induced to produce smutted panicles, although the Nuda group has always been considered very susceptible.

Resistance in wheat seems to be recessive but dominant in oats. Both can best be interpreted on the basis of multiple factors.

The control of loose smuts of wheat and barley, and barley stripe by Uspulun, Semesan, and Germisan. H. A. RODENHISER and E. C. STAKMAN.

Uspulun (0.25 per cent sol.), Semesan (0.3 per cent sol.), and Germisan (0.25 per cent sol.) controlled loose smuts of wheat and barley, and barley stripe when the seed was soaked at 45° C. for one hour or longer. About seven per cent of heads in control plats were smutted, while there was only a trace of smut in the treated plats. The percentage of plants affected with stripe was reduced from thirteen per cent to less than one per cent. Germisan also practically eliminated stripe when seeed was soaked for three hours at ordinary temperatures. Shorter periods of immersion were not effective. Hot Germisan injured the seed slightly.

A leaf-spot of redtop caused by an apparently undescribed species of Helminthosporium. CHARLES DRECHSLER.

During July, 1923, in the vicinity of Washington, D. C., the foliage of redtop (*Agrostis alba* L.) was found spotted with elongated dead regions dull reddish-gray in color. At first small, these lesions later often attained a length of over 10 centimeters, thus involving considerable portions of the leaf blade. From the central portions of the affected regions, conidiophores typical of Helminthosporium were found to emerge, bearing straight, cylindrical, olivaceous, 1-to-9 septate conidia, germinating by the production of lateral and oblique germ tubes from end and intermediate segments. In pure culture, sporulation in moderate quantity may be obtained, often with meager development of aerial mycelium, and production of numerous small sclerotia, thus in the latter details differing from the congeneric parasite causing purple leaf-spot of oats, the conidial condition of which it closely resembles. It differs from *H. siccans* Dr. in the inferior dimensions of its conidia and in pathological habit. The fungus appears to be of widespread distribution, having been previously collected on Long Island and in Connecticut, the collections, however, lacking clear evidence of parasitic relationship owing to frequent withering of redtop foliage from other causes.

Relation of internal cob-discoloration to yield in corn. (*Second progress report*). R. A. JEHLE, F. W. OLDENBURG, C. E. TEMPLE.

Seed corn was carefully selected for quality and type. The butts and tips were chopped off, and the seed ears were divided into at least two lots, those which showed red, gray and other abnormal internal cob-discoloration, and those which were practically free from internal cob-discoloration. Yield records were obtained from plots grown from each lot. These records show an apparent correlation between internal cob-discoloration and yield, which was more pronounced on light sandy and clayey soils than on the best corn land. This is indicated in the following table:

Year	No. of tests	No. of counties	Av. yield of corn from discolored cobs	Av. yield of corn from cobs with no discoloration	Average increase bushels	Average increase per cent
1921 Total	6	4	39.57	51.46	11.89	23.1
1922 Total	39	16	57.86	67.19	9.33	16.12
1923 Total	24	13	56.80	63.58	6.78	12.00
1924* Total	16	11	45.20	53.59	8.39	18.56
1921 VG**	1	1	60.4	63.3	2.9	4.8
1921 MP***	5	4	35.41	49.13	13.72	38.77
1922 VG	9	3	84.16	88.81	4.65	5.5
1922 MP	30	15	51.39	60.86	9.47	18.43
1923 VG	3	2	78.25	80.25	2.00	2.55
1923 MP	21	11	53.74	60.99	7.25	13.50
1924* VG	2	2	61.57	63.45	1.88	3.05
1924* MP	14	10	42.86	52.17	9.31	21.95

*(1924) Record incomplete, **(VG) Very good corn land, ***(MP) Medium to poor land.

Foot and root rots of wheat in Minnesota in 1924. J. J. CHRISTENSEN and E. C. STAKMAN.

In Minnesota in 1924, foot and root rots killed from 2 to 4 per cent of the plants of common wheat, and from 5 to 15 per cent of plants of durum wheats after heading. Seedling injury is not included. In many fields of durum 20 to 30 per cent of plants were killed, and in several as many as 75 per cent succumbed. More than seven hundred isolations were made from diseased plants. *Helminthosporium sativum* was obtained from 71 per cent of the platings; Fusarium spp. from 22 per cent, and other fungi from 7 per cent. One hundred and four varieties were grown on sick soil at University Farm, St. Paul. These included durum, club, emmer, spelt, poulard, polish, and vulgare wheats. All species and varieties were attacked, but in different degrees. The durums were most susceptible. The average percentage of killing in fourteen varieties of durum was 78; and in seventy-one varieties of vulgare wheats, 9.9 per cent. The average percentage of killing for all varieties was 20.1.

The control of flax rust. A. W. HENRY and E. C. STAKMAN.

Flax rust (*Melampsora lini* (Pers.) Desm.) may practically ruin a crop for fiber purposes and may also reduce the yield of seed. Several immune or highly resistant seed-flax varieties have been found. Numerous strains of large-seeded, blue-flowered Argentine flax are immune and some also are wilt resistant. Ottawa 770B, a yellow-seeded, white-flowered variety, also is apparently immune. Several other seed types are either resistant or immune. Rust resistance and wilt resistance are not necessarily correlated. Winona is wilt resistant but susceptible to rust, and rust-immune strains of Williston Golden are particularly susceptible to wilt.

As our good fiber varieties like Saginaw, and seed varieties like Winona, are susceptible to rust, these have been crossed with the immune seed types. Rust resistance appears to be dominant, and segregation in the F_2 indicates that immunity can be combined with the desired morphological characters.

No infection of seedlings resulted from inoculating flax seeed with viable urediniospores before sowing, but successful infection did occur when bits of telia were mixed with the seed. Obviously thorough cleaning of the seed is advisable. Crop rotation and early seeding are also important preventive measures.

(Cooperative investigations between the Office of Cereal Investigations and Office of Fiber Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Factors affecting the development of Melampsora lini (Pers.) Desm. HELEN HART.

Aeciospores and urediniospores of *Melampsora lini* (Pers.) Desm. germinate in distilled water at temperatures ranging from 0.5° C. to 27° C., the optimum being about 18° C. They germinate equally well in light and darkness. Host tissue did not stimulate germination appreciably. Teliospores require a rest period which could not be shortened artificially. Urediniospores may begin to germinate within one and one-half hours and only three hours in the moist chamber are required for infection. Uredinial germ tubes enter through the stomata of resistant as well as susceptible varieties. Under favorable conditions uredinia are formed in about nine days. Light hastens their formation, while darkness retards it. Uredinia are formed at temperatures ranging from 7° C. to 30° C. The effect of nutrients is indirect, intensity of infection being directly proportional to luxuriance of growth of the host. Rust from common flax infected *Linum rigidum* but not *L. lewisii*, while that from *L. lewisii* did not infect common flax.

(Cooperative investigations between the U. S. Department of Agriculture, Bureau of Plant Industry, Office of Cereal Investigations, and the Minnesota Agricultural Experiment Station.)

Alternate hosts of Puccinia coronata Corda. S. M. DIETZ.

In a further study of the alternate hosts of *Puccinia coronata* Corda, the response of the four genera, Berchemia, Ceanothus, Zizyphus, and Rhamnus, of the family Rhamnaceae, has beeen determined. *Berchemia scandens* produced aecidia when exposed to infection from teleutospores on *Avena sativa*. *Ceanothus americanus* and *Zizyphus lycioides* gave negative results with the specialized forms on Avena, Calamagrostis and Festuca.

The response of five additional native and two European species of Rhamnus was also determined. *Rhamnus dahurica*, *R. ilicifolia*, *R. pachyphylla*, *R. pinetorum*, *R. rubra*, and *R. tinctoria* produced aecidia with the specialized form, Avenae. *Rhamnus pinetorum* and *R. rubra* produced aecidia with the specialized form on *Calamagrostis canadensis*, while only pycnidia were produced on *R. dahurica*, *R. pachyphylla*, and *R. tinctoria*. Teleutospores on *Festuca elatior* produced aecidia on *R. rubra*. Although *R. nevadensis* gave negative results from teleutospores on all three gramineous hosts, further trials are necessary to firmly establish its resistance.

No increase in degree of infection on *Rhamnus frangula* during 1923 and 1924 was obtained. Intensive field surveys conducted in 1923 and 1924 disclosed no aecidial infection, thus corroborating the earlier suggestion that *Puccinia coronifera* Kleb. is absent from America.

Although the genus Lepargyraea was exposed to infection with teleutopores on Avena, Calamagrostis, and Festuca, only *L. canadensis* produced aecidia from teleutospores on *C. purpurascens*.

(Cooperative investigations by the Iowa Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

The inheritance of resistance to Puccinia graminis avenae. S. M. DIETZ.

Inheritance of resistance to *Puccinia graminis avenae*, Form 2, was studied by artificially infecting the F_1, F_2, and F_3 generations of crosses between eight pure-line varieties of oats, studied from 1919 to 1923 under field and greenhouse conditions, using percentage of infection and size of uredosori as a measure of resistance or susceptibility. Resistance was dominant and based on a single factor difference in the National × White Tartar and Lincoln × White Tartar crosses.

Three genetically-different susceptible Burts were found. At least two factors, one an inhibitor, were involved. In a Burt × White Russian cross, the F_1 was susceptible and the F_2 segregated into 58 resistant to 251 susceptible plants. The resistant F_2 plants segregated into one homozygous resistant to two heterozygous families. In the second Burt × White Russian cross, the F_1 was susceptible, the F_2 segregated into 23 resistant to 77 susceptible plants. In the third Burt × White Russian cross the F_1 was resistant and the F_2 segregated into 185 resistant to 45 susceptible plants.

The F_2 of Iowa 105 × Green Russian and Ruakura × White Russian segregated into 300 resistant plants to one susceptible.

(Cooperative investigations between the Iowa Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

Varietal tests of peanut (Arachis hypogaea) for wilt resistance. CARL HARTLEY.

Tests were conducted, in Java, of the resistance of various peanut populations to bacterial wilt (*Bacterium solanacearum*). African, South American, and North American varieties, particularly Valencia, proved more susceptible at Buitenzorg than the best Javanese varieties. The local types, in order of decreasing resistance, are Tjina, Holle, and Brol. Within the important Holle type great differences in resistance were found between different populations. The disease is very irregularly distributed in the fields, and numerous replications were necessary to establish the significance of some of the differences observed. Thirty-foot rows were used, so arranged that each variety occurred adjacent to each other variety in one replication of a series, and one only. Practically all plants on sick soil become infected, and the percentage surviving proved the only practicable criterion of relative resistance. This is an index rather than a true measure, resistance being incommensurable. The usual probability methods could not be directly applied to the results, and the significance of differences between two varieties was judged by the proportion of the replications in which one variety surpassed the other. Decided improvements in resistance were quickly obtained in some populations by mass selection. The work is being continued by Dr. Marie B. Schwarz, at the Instituut voor Plantenziekten, employing line-selection methods.

Two new bacterial diseases. MEL T. COOK.

A wilt disease of the egg-plant becomes evident with the formation of the fruit, causing a wilting and dying. When the young plants are inoculated they do not show symptoms of the disease until the fruits are forming. Bacteria are found in great abundance in the vascular tissue. The organism will not attack tomato, pepper or tobacco.

A bacterial disease of cultivated Cosmos becomes evident at almost any period during the life of the plants, causing them to wilt and die. The vascular tissue contains an abundance of bacteria which will reproduce the disease when inoculated into healthy plants. A Fusarium is usually associated with the bacteria but it will not cause the disease. The organism is different from the organism attacking the egg-plant.

The control of white pine blister rust in the Northeastern states. E. C. FILLER.

A resume of this subject up to the time of the Cincinnati meeting was printed in Phytopathology 14: 53, 1924. The speaker was unavoidably absent. The present paper brings the subject up to date.

Presence of the European brown-rot fungus in America. WALTER N. EZEKIEL.

Isolations from fruits from California, and the spur-blight Monilia from Oregon, were identified as the true European brown-rot fungus, *Sclerotinia cinerea* (Bon.) Schroeter. This can be distinguished morphologically by numerous mycelial characters in drop cultures, as well as culturally, from *S. americana* Norton and Ezekiel, which is the species occurring predominantly in this country.

Monilia oregonensis Barss and Posey is probably synonymous with *S. cinerea*, agreeing with it morphologically, culturally, and in its life history.

S. cinerea is expected to be of slight economic importance in this country as compared to *S. americana*.

The importance of removal of mummies and affected fruit in apple bitter-rot control. R. H. HURT.

Observations on the spread of bitter-rot in Virginia apple orchards in 1923 indicated the removal of mummies and affected fruits as valuable procedures in control. These practises were followed in 1924 in an orchard of the Yellow Newtown variety. In one block of 5 trees all mummies were removed during the dormant season. In a second block of 15 trees, affected fruits and all mummies observed were removed at weekly intervals during July and August. Three trees served as controls. All of the trees received a single application of Bordeaux mixture July 17. The results are recorded as follows:

	Mummies removed	Mummies and affected fruits removed	Controls
Number of apples	4911	20,925	4203
Number bitter-rot	236	180	2089
Percentage bitter-rot	4.81	0.86	49.7

The time expended in removal of mummies and affected fruits in the 15-tree block averaged about eleven minutes per tree for the season. On this basis the time expended per acre per season would be approximately eight hours.

Rainfall in relation to ascospore discharge and infection in Venturia inaequalis. F. J. SCHNEIDERHAN.

Ascospore discharge studies of the apple scab fungus have been made at Winchester, Virginia, during three consecutive years. The rainfall of the critical months during the three years has been extremely varied and the variations are strikingly correlated with intensity and duration of ascospore discharge and with intensity of infection. Discharges occurred only during rainfall and the majority of rains within the period of discharge were accompanied by spore discharges. The number and distribution of the discharges during the critical months of the three years together with the length of the discharge periods are recorded as follows:

Year	April	May	June	July	Total	Discharge period (days)
1922	4	9	3	0	16	56
1923	1	4	5	3	13	94
1924	3	7	4	0	14	61

Percentages of apple fruits affected with scab on unsprayed trees within the same small block during the three years are as follows:

Year	Stayman	Winesap	Rome
1922	76.0	97.3	98.4
1923	1.3	7.6	4.2
1924	67.5	77.5	- -

May rainfall apparently determines to a high degree the intensity of infection for the season. The rainfall of this month was 3.63, 1.12, and 10.75 inches for 1922, 1923, and 1924 respectively.

The seasonal development of apple scab. F. J. SCHNEIDERHAN.

Studies of the incidence of scab on apple fruits during 1924 show three infection cycles during the early season, cessation of spread in midsummer, and renewed activity in the fall. Ascospore inoculum applied May 12 to fruit buds of Early Harvest in the late pink stage produced fruit lesions on May 28. Secondary lesions from conidia, carried by drip and wash of rain, appeared June 17 to 19. This cycle represented a gradual curve with the peak on the dates indicated. The third series of lesions represented a sharper curve with the peak on July 1. Studies on Winesap and Stayman showed no further spread of infection during a drought from early July until late August, when infection was resumed following rainfall, and a continuous slow spread, with no clearly defined cycles, occurred until harvest, October 17. A progressive decrease in the rate of enlargement of scab lesions occurred throughout the season. The initial lesions attained an average diameter of 9 mm. within a month, while those which appeared towards harvest were of the "pin head" type and the rate of enlargement was scarcely measurable. Slight, but measurable, enlargement occurred on ripe apples under bell jars in the laboratory.

Frequencies of ascospores of Venturia inaequalis in orchard air. G. W. KEITT and L. K. JONES.

Earlier studies by Frey and Keitt have been continued and extended. Orchard air was drawn by means of a motor-driven suction apparatus through a suitably arranged filter of nitrocellulose and thence through a gas meter which recorded the volume. At the end of the run the nitrocellulose filter was dissolved in a suitable straight-walled, flat-bottomed glass container, in a mixture of alcohol and ether, and allowed to evaporate to a thin, tough, pliable, transparent film. The number of ascospores caught was computed from microscopic counts. This is an adaptation of a technique used by Pasteur. It appears promising for wider applications.

At Sturgeon Bay the apparatus was run in an apple orchard throughout the period of ascospore discharge. The filter was eighteen inches above ground, protected from rain by a shelter which permitted free passage of air. Ascospores were caught at intervals from May 6 to June 29 (apples bloomed June 2–18). The maximal occurrence of ascospores was recorded May 13, when during a rain they were caught through a four-hour period at the average rate of 289 per cubic foot of air. The major portion of the season's discharge occurred before the blooming period.

Further studies of the seasonal development and control of apple scab and cherry leaf spot. G. W. KEITT and L. K. JONES.

The developments of the past season have emphasized the contrasts previously reported by the writers in the seasonal development and control of apple scab and cherry leaf spot. At Sturgeon Bay ascospores of *Venturia inaequalis* were being discharged when the cluster buds of the apple began to open. Infection began in very early stages of unfolding of cluster buds, about 20 days before the "pink" spray was applied. On Dudley plots, where perithecia of the fungus were very abundant, 85 per cent of the blossoms developed sepal infection. Fifty-eight per cent of the blossoms showed sepal infection before the "pink" spray was applied. These results emphasize the importance of sanitation where feasible, and of a "prepink" fungicidal treatment. Under extreme conditions one "prepink" treatment may be insufficient.

Although ascospore discharge of the cherry leaf spot fungus began by May 14 and discharges occurred frequently thereafter, no disease was observed until June 25.

Studies showed that this infection was occasioned by ascospores discharged June 15. Although numerous moist periods occurred in early spring, infection by the cherry fungus was delayed until higher temperatures prevailed, little injury occurring even on unsprayed trees before harvest. Later, however, unsprayed trees were severely infected.

Longevity of the uredospores, teliospores and sporidia of Cronartium ribicola. PERLEY SPAULDING and ANNIE RATHBUN-GRAVATT.

The latter parts of the summers of 1923 and 1924 have been spent in a study of the longevity and viability of the teliospores and sporidia of *Cronartium ribicola* produced naturally upon *Ribes americanum, R. cynosbati, R. glandulosum, R. nigrum, R. odoratum, R. rotundifolium, R. triste* and *R. vulgare.* Incidentally data upon the longevity of the uredospores were secured. Freshly matured teliospores seem to possess equal powers of germination irrespective of the host species upon which they were produced. Their longevity was found to vary from 19 days for one collection of *R. rotundifolium* to 87 days for *R. nigrum,* which was still germinating strongly at the end of the experiment. The "floating" method of germination was found by test to be best. The germination period for teliospores increases directly with their age.

The sporidia need free water to germinate, but the thinnest film is sufficient. When exposed dry upon glass slides, in air with a rather high moisture content, they survived and gave fair germination at all periods of time ranging from 30 minutes up to 26 hours, which was the longest time that a test was run. They survived alternate drying and wetting four and five times quite well and even up to eleven times in scanty numbers. Icing of the teliospores does not appear to have a decided stimulating effect; icing of the sporidia does appear to have a decided stimulative effect in some cases.

Studies on the tip burn disease of lettuce. A. G. NEWHALL.

An investigation has been made of the relation of certain fertilizers, temperature, sunshine, and soil moisture to this disease under field and greenhouse conditions, using Big Boston head lettuce. Osmotic pressure and catalase activity have also been studied. It is concluded that the disease is not of bacterial origin, though bacteria may play an important secondary role. Fluctuations in temperature and moisture supply, particularly in the presence of readily available potassium and nitrogen, induce tip burn. Conversly, slowing down the rate of growth by leaving potash out of the fertilizers and by root pruning or deep cultivation at the proper time have reduced the amount of the disease. Slow growing varieties of head lettuce were found to be less subject to tip burn.

Rye resistant to leaf rust, stem rust, and powdery mildew. E. B. MAINS.

In 1921 two plants of Abruzzes rye highly resistant to leaf rust, *Puccinia dispersa,* were found also to be practically immune from powdery mildew, *Erysiphe graminis secalis.* The next year 7 plants from selfed seed [illegible] 277 from open-pollinated seed were studied for susceptibility to leaf rust [illegible] mildew and the most promising of these for susceptibility to stem rust, *Puccinia graminis secalis.* The cultures of leaf rust and stem rust were developed from single spores. All types of susceptibility from high resistance to high susceptibility to each of these diseases were found among these plants. Selfs and crosses in this material were studied the next year. From these studies it appears that resistance to each disease is dominant. Plants were found showing resistance to one or more of the diseases and susceptibility to the others, indicating that resistance to each is due to separate factors independently inherited. Many, however, were resistant to all three diseases. The low self fertility of rye and the dominance of resistance have prevented thus far the certain isolation of a pure strain resistant to all three diseases.

(Cooperative investigations by the Purdue University Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

Soil-inhabiting fungi parasitic upon the pea plant and their relation to disease. FRED REUEL JONES.

The soil-inhabiting fungi parasitic upon the pea plant which have been studied in cooperation with the University of Wisconsin may be conveniently divided into three groups based upon the portion of the plant into which the fungus makes its most significant invasion. The first group consists of those species which attack chiefly the growing ends of shoots and roots. The important members are *Corticium vagum* and two species of Pythium. Although thoroughly distributed, they cause important injury only occasionally under favoring conditions.

The second group includes those species entering chiefly the base of the stem near the attachment of the cotyledons of the seed. This is a numerous group consisting largely of species of Fusarium. No species in this group has been found sufficiently cumulative under usual field conditions to cause independently important crop reduction, even with intensive pea culture.

The third group consists of two species entering and traversing extensively the primary cortex of roots. They are a mycorrhizal fungus and *Aphanomyces euteiches* Drechsler. The former appears to be of no economic consequence in peas; the latter is the most widespread cause of destructive root-rot.

Infection of barley by Ustilago nuda through seed inoculation. W. H. TISDALE and V. F. TAPKE.

Floral infection of barley by *Ustilago nuda* has for many years been accepted as proved.

Investigations by the Office of Cereal Investigations, U. S. Department of Agriculture, have shown that dehulled seed of Han River Tennessee Winter, Wisconsin Winter, Greece, Alaska and Texas Winter barleys, inoculated with spores of *U. nuda,* produce plants with high percentages of loose smut. Nakano Wase remained smut-free, even though the seed was dehulled and smutted. Seedlings from dehulled, inoculated seed of all varieties studied were severely injured and many failed to emerge. Seedlings from dehulled inoculated seed, sown ¾ of an inch deep, emerged in higher percentages than those from similar seed sown 1½ inches deep. Seedlings from inoculated seeds with the hulls not removed, were not noticeably injured but some of them were infected. Spores of *U. nuda,* devitalized by pasteurization, were not harmful to seedlings grown from the inoculated seed. Neither viable nor devitalized spores of *U. nuda* were harmful to seedlings of Red Wave wheat grown from inoculated seed.

A microscopic study revealed infection of the coleoptile and first leaves of the plumule of seedlings of both the susceptible varieties and the resistant variety Nakano Wase by *U. nuda.*

Influence of balanced nutrient supply on susceptibility of corn plants to Gibberella saubinetii (Mont.) Sacc. G. N. HOFFER and J. F. TROST.

The relative extents of injury to three strains of corn by infection with *Gibberella saubinetii* (Mont.) Sacc. were varied by supplying young plants of each strain with controlled supplies of mineral nutrients added to washed sand. The basic sand culture contained the proportions of nutrients in the Hartwell and Pember solution. Three other sets carried the basic nutrient proportions excepting that the phosphorus and potassium contents, respectively, were reduced to one-tenth of the basic culture.

Seeds of Burr-Leaming, single-crossed Leaming, and single-crossed Burr-White were supplied by D. F. Jones. Triplicate sets of six uninoculated seeds of each strain and six seeds inoculated with a spore suspension were planted. The plants were grown for five weeks at 20° C. and at uniform moisture in temperature tanks.

The percentage reductions in dry matter per plant for each nutrient treatment are as follows:

Treatment	Strain			Average for three strains
	Burr-Leaming	F_1-Leaming	F_1-Burr-White	
Basic solution	16.5	—2.0	17.1	10.5
Deficient phosphorus	55.6	43.8	14.0	37.8
Deficient potash	26.3	51.3	22.8	33.5

When plants grown under conditions where either phosphate or potash was deficient, injuries induced by *Gibberella saubinetii* were markedly different for each respective strain.

(Cooperative investigations by the Purdue University Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

EUNICE [illegible]CKWOOD OBERLY

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EUNICE ROCKWOOD OBERLY

1878–1921

NEIL E. STEVENS[1]

WITH PORTRAIT. WITH PLATE II

"The librarian who builds up the collection for a research institution, and in connection with it, administers an effective bibliographical service, is making a fundamental contribution to the research work of that institution, too important to be underestimated or forgotten, merely because he may not find time to publish catalogs or bibliographies." These words, inserted as an afterthought in the paper read before the June 1921 meeting of the American Library Association by Miss Eunice R. Oberly express with characteristic brevity and directness Miss Oberly's appraisal of library work. They also express the attitude of her associates, whether librarians or investigators, toward her own work. This feeling of appreciation has found concrete expression in the establishment of the prize to which reference has just been made. This prize is an especially appropriate memorial. Miss Oberly's best memorial, however, is her record of sound achievement and enthusiastic service, only a brief outline of which will be attempted here.

Eunice Rockwood Oberly was born on March 7, 1878, in Cairo, Illinois. Her father, John H. Oberly, was at the time editor of the "Cairo Bulletin." She was the youngest of seven daughters. Her family moved to Washington in 1885 (her father serving as Civil Service Commissioner and later as Commissioner of Indian Affairs in the Cleveland Administration) and it was here that Miss Oberly spent the greater part of her life. She was prepared for college in the public schools of Washington and at Concord, New Hampshire, her father being for a short time editor of the "Patriot," published in Concord. She was graduated from Vassar in June, 1900, being class historian. She came to the U. S. Department of Agriculture in the summer of her graduation from college and, after a few years of bibliographical work in the Bureau of Animal Industry, she was appointed librarian of the Division of Vegetable Physiology and Pathology. With the consolidation of the library of the Division of Vegetable Physiology and Pathology with the library of the Office of Botanical Investigations in 1908 to form the library of the Bureau of Plant Industry, she was made librarian of the bureau, which position she held at the time of her death. Her whole professional life was, therefore, spent in the U. S. Department of Agriculture.

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[1] The preparation of a suitable biographical sketch of Miss Oberly's life has been unduly delayed for unavoidable reasons. It is felt that the awarding of the first prize in her name is an appropriate occasion for publishing such a sketch, which has just been made possible by Dr. Stevens.—Editor.

The "Plant Industry Library" was and still is a library without books. It is essentially a library service for the purpose of indexing and cataloging botanical literature and making readily available to the workers in the Bureau of Plant Industry the literature in the libraries of Washington and elsewhere, especially, of course, the Library of the United States Department of Agriculture and the Library of Congress.

There is little opportunity in such a library to develop the attitude of a "curator" of books so often found in university librarians and so irritating to university investigators. Probably in any environment Miss Oberly's ideals and methods would not have been very different. Certainly in the library of the Bureau of Plant Industry her dominant idea was that the library should be of the greatest possible service to the investigators. In almost every one of her various statements of the aims and methods of her work occur, "Service is the watchword of the modern librarian," and "The book which any person may want when he wants it." How well these ends were attained in the administration of her own library is abundantly attested by the fact that the organization which she built up was regarded by botanical investigators in Washington as almost a model of its kind. Of the Bureau of Plant Industry Library, botanists in university centers always spoke with respect, often with envy. A few years ago the writer published in Science a note under the title "The Obligation of the Investigator to the Library." This brought a somewhat heated response from a professor of botany in one of our great universities, the general tenor of which was that not all libraries lived up to the ideals which the writer had taken for granted. This letter concluded "Your library is one of the striking examples of subordination of library autonomy to the researchers."

As was natural, in view of the large amount of work in the study of plant diseases carried on in the Bureau of Plant Industry, Miss Oberly's special contributions to bibliography were on the subject of phytopathology. The list of literature on plant diseases printed in "Phytopathology" from June, 1914, to December, 1920, was begun by her, although afterwards carried on by members of her staff. Another example of her cooperation with the activities of the Department was the work of her library in editing and standardizing the bibliographies in the Bureau of Plant Industry publications and in the "Journal of Agricultural Research." Her knowledge of the relations and organization of phytopathological literature was probably unique, and the card catalog on this subject which she initiated became of great service. Its wide use and appreciation by the Bureau of Plant Industry pathologists prove its merit. The "Check List of Publications of the Department of Agriculture on the subject of Plant Pathology, 1837–1918," which was issued in 1919 as No. 1 of the Bibliographical Contributions of the Department of Agriculture Library, was prepared by Miss Oberly, although her name does not appear on it. The "Check List of Publications of the State Agricultural Experiment Stations on the subject of Plant Pathology," issued as Bibliographical Contribution No. 2, was begun by her but was completed after her death by her assistant, Miss Jessie M. Allen. Two bibliographical articles which she prepared in the summer of 1921 were also published after her death, namely, "The Contributions of Librarians to Agricultural History and Research," which appeared in the "Library Journal" for March 15, 1922, and an article on "Abstracts and Titles of Scientific Articles from the Librarian's Standpoint," published in "Science" for November 18, 1921. Both of these articles show her deep interest in scientific bibliography.

It was Miss Oberly's conviction that it would be of great advantage both to investigators and librarians if there could be developed, in some way, greater cooperation between them. At the time of her death she was at work on a plan whereby the librarians of research institutions might identify themselves with the scientific societies as well as with the library associations, and had even suggested the possibility of a bibliographical section in the American Association for the Advancement of Science.

Miss Oberly's interest was thus by no means confined to making her library of use to investigators in Washington. It was her ambition to make the combined library facilities of the country more readily available to the botanical investigators of this country and indeed of all countries. To this end she advocated a census of the library facilities of the country, which should be available to librarians and investigators alike.

The results of the cataloging and indexing of botanical literature carried on in the Bureau of Plant Industry were through her efforts made directly available to botanical workers outside of Washington in two ways. From 1914 to 1920 there was furnished for publication in "Phytopathology" a monthly list of literature relating to plant diseases. Later there was developed the circulation from the Bureau Library of a mimeographed list of botanical literature. This list, which is issued every two weeks, contains full citations of all important botanical literature received by Washington libraries. As a means of keeping investigators in constant touch with the new literature of their subject, this list has never been equalled. It proved immensely popular; requests for it and letters of appreciation were received from botanists all over the United States, so that now more than two hundred and fifty copies are regularly sent to American investigators and institutions outside Washington. There is also a small foreign circulation, and Dr. E. J. Butler, Director of the Imperial Bureau of Mycology, in England, stated during his visit to this country in 1921 that this list of literature was the most useful thing that the Bureau received from the Department of Agriculture.

Of Eunice Oberly's rare qualities of mind and of her vivid and attractive personality it is unnecessary to write in a journal, a large number of whose readers were her friends. To readers who did not know her personally, any terms the writer might use would certainly seem extravagant. Ten years ago, when the writer entered the Bureau of Plant Industry, he was told by the head of an office that you could argue with Miss Oberly just as you would with a man. This was intended as a great compliment and apparently meant that opposition to her own opinion produced no irritation and that she showed extraordinary patience, tolerance, and fair mindedness toward the opinions of others. The resolutions adopted by the American Phytopathological Society at its first meeting after her death certainly expressed the feeling of the membership, as well as the committee, in referring to her as a colleague and friend, and acknowledging the debt of the society as an organization and the membership as individuals to her "kindliness, efficiency, and wide acquaintance with the literature of our subject." This wholly inadequate appreciation of Miss Oberly's work will probably be best concluded as it was begun by quotation from the paper written a few months before her death on "Contributions of Librarians to Agricultural History and Research," which gives in her own words the ideals to which her professional life was devoted.

"Hand in hand with the discovery and publication of new facts should go the constant development and perfection of our means for discovering the facts recorded in the mass of scientific literature already published. The necessity of division of labor here is obvious, though for the best results there must always be in this work the closest cooperation between librarian and research workers. The opportunities for agricultural research librarians and bibliographers to render service, at present, perhaps, undreamt of, are limited only by the appreciations of these possibilities on the part of research workers. The more encouragement librarians receive, the more eagerly will they undertake to qualify themselves to increase a hundredfold their efforts to mobilize and organize the literature of agriculture for the use of the research worker, thus conserving his time for original observation and experiment in the field and laboratory for which he is trained."

FIRST AWARD OF THE EUNICE ROCKWOOD OBERLY MEMORIAL PRIZE

NEIL E. STEVENS

Announcement of the first award of the Eunice Rockwood Oberly Memorial Prize was made the last of December, 1924, during the Washington meetings of the American Phytopathological Society. The prize was awarded to Mr. Max Meisel, formerly of the Science Division of the New York Public Library, for the first volume of his extensive bibliography on American natural history, published in the fall of 1924 by the Premier Publishing Company, 626 Broadway, Brooklyn, N. Y. Its scope is explained in the full title of the bibliography which is as follows: "A Bibliography of American Natural History: The Pioneer Century, 1769–1865; The role played by the Scientific Societies; Scientific Journals; Natural History Museums and Botanic Gardens; State Geological and Natural History Surveys; Federal Exploring Expeditions, in the rise and progress of American Botany, Geology, Mineralogy, Paleontology and Zoology." It is to be published in three volumes. The first volume contains 244 pages and is "an annotated bibliography of American natural history and its institutions, during colonial times and the pioneer century, which have been published up to 1924; with a classified subject and geographic index; and a bibliography of biographies." This bibliography will be a very useful reference tool and sets a high standard of comparison for material which may be submitted in competition for the Oberly Prize in the future.

The foundation of this prize in memory of Miss Oberly, formerly Librarian of the Bureau of Plant Industry of the U. S. Department of Agriculture, is the direct outcome of the spontaneous expression of regard by her associates at the time of her death. Her sudden death on November 5, 1921, after only a few days' illness was a great shock to her friends and co-workers. From all of them contributions for flowers poured in without solicitation until the amount was so large that it could not appropriately be spent on so perishable a memorial. Thus within a few hours of her death plans were started looking toward the establishment of a permanent memorial which would not only perpetuate her memory but also help in carrying forward the work in which she was so deeply interested.

From among friends in the U. S. Department of Agriculture who were closely associated with Miss Oberly in her work, a temporary committee was formed to handle the contributions and to decide on the form of the memorial. At first, contributions to the fund were received only from Miss

Oberly's associates in the Department of Agriculture, who gave about two-thirds of the first amount received. Later, contributions were received also from friends and library co-workers outside of the Department, from her college classmates and from the American Phytopathological Society. The amount of the contributions soon reached the goal of the temporary committee, which was $1,000, but it is hoped and expected that the amount may be increased from time to time.

In view of Miss Oberly's own bibliographical contributions and her appreciation of and belief in the possibilities of service inherent in bibliographical work, the committee unanimously decided to have the memorial take the form of a cash prize to the amount of the annual or biennial interest on the $1,000, to be awarded to the compiler of the best bibliography in the field of agriculture or the natural sciences. It was further decided to ask the American Library Association to administer the fund and to appoint a permanent committee with power and authority to formulate rules and conditions to govern the award of the prize and to select and designate the beneficiary.

The administration of the fund was definitely accepted by the American Library Association in December, 1922. The contributions to the fund were turned over to the American Library Association by the temporary committee on March 31, 1923. The President of the American Library Association then appointed the permanent Eunice Rockwood Oberly Memorial Fund Committee with the following membership: Claribel R. Barnett, Chairman, Librarian, U. S. Department of Agriculture, Washington, D. C.; William Warner Bishop, Librarian, University of Michigan, Ann Arbor, Michigan; Mary E. Hazeltine, Preceptor, University of Wisconsin Library School, Madison, Wisconsin; Mary G. Lacy, Librarian, Bureau of Agricultural Economics, Washington, D. C.; Edward D. Tweedell, Assistant Librarian, John Crerar Library, Chicago, Illinois; Mary K. Bryan and Erwin F. Smith, Bureau of Plant Industry, Washington, D. C.

SUSCEPTIBILITY OF NICOTIANA SPECIES, VARIETIES AND HYBRIDS TO TOBACCO WILDFIRE

P. J. Anderson

The origin of tobacco wildfire is an unsolved riddle. It suddenly appeared in North Carolina in 1916 and in a few years has spread to all tobacco sections of America. But where was it before 1916? Nobody knows. It is known that many pathogenes which suddenly appear and ravage a cultivated crop lived originally on some related wild host. But when the writer began working on this disease four years ago, wildfire had never been found on other host plants. He therefore started a series of infection experiments to see whether the disease would affect other plants besides cultivated tobacco. In a quest of this kind one first suspects the different varieties of the same species which has the disease, then the different species of the same genus, then species of the same family. Although a few species outside the genus Nicotiana have been tested, the bulk of the work up to the present has been within this genus. This paper deals only with results of inoculations within the genus Nicotiana.

There was also a more directly economic reason for undertaking the investigation. It was hoped that some resistant varieties of tobacco or species of Nicotiana might be found which would serve as a basis for development of a desirable resistant kind of tobacco.

METHODS

The time-consuming phase of this work was the collection of seed of a large number of species and varieties. For many of these I am especially indebted to Dr. R. E. Clausen of the University of California and Dr. J. J. Johnson of the University of Wisconsin. Most of the others were secured from the seed houses in Germany, France, and England. Nicotiana is a genus of about 40 species but, up to the present, seed of only 20 species has been secured. Varieties and strains of the species *N. tabacum* are so numerous that one hardly hopes to test all of them, but the group which was tried is probably fairly representative of the species. Seed of all species and varieties was sown in plots in tobacco beds in the usual way in which tobacco is started in the Connecticut Valley. Inoculations were begun as soon as the leaves were as large as the fingernail. Inoculation was repeated every day or two for four weeks or more. The method of inoculation was one which has been successfully used by the writer for several years in securing a high percentage of infection in tobacco beds on a large scale:

A young wildfire spot on a tobacco leaf is cut out, sterilized, washed and transferred to a flask of nutrient bouillon. As soon as the bouillon has clouded—24 to 48 hours—it is poured into a sprinkling can of water and the water sprinkled over the plants in the bed. The inoculations are made in the evening in order that the infested drops of water may remain on the leaves over night before drying down. The same results may be accomplished by inoculating the flasks from a pure culture each day, but it is the writer's impression that this has not resulted in as thorough infection as when the strain was isolated from a fresh lesion every day. Of course, one cannot be sure that his flask cultures are always pure cultures of *B. tabacum,* but since it is hardly probable that any other disease would be confused with wildfire, the chance of error from this source is negligible. Where it was necessary to inoculate several square rods of bed every day, this method could be used with the minimum amount of labor. In numerous trials with *N. tabacum,* this method has given close to 100 per cent. of infected plants, and frequently the plants were so covered with lesions that large numbers of them died. The plants were never previously wounded by needle pricks or other artificial means and no bell jars were kept over them. They were kept as nearly as possible under the conditions which a tobacco grower would maintain for his beds. These latter points should be kept in mind in comparing the results which the writer obtained with those recently reported by Johnson, Slagg and Murwin.[1] Their tests were with plants grown in pots in the greenhouse, and their list of susceptible species is based on results secured by first puncturing the leaves with needles and then keeping the plants in a moist chamber for one or two days after inoculation. In this way they were able to infect all species and varieties tried, but state that "fairly marked differences occurred when the organism was applied by the atomizer only," *i.e.,* without puncturing the leaves.

There were at least several hundred, and frequently more than a thousand seedlings in the plots of each species and variety tested by the present writer. The tests have been repeated for most of them at least once during the work. No attempt was made to count the number of infected plants or the number of lesions, because the work involved would be out of proportion to the value of the results. Susceptibility was rated by periodical observation and estimation of the severity of infection as compared with that of a Havana strain of *N. tabacum* which was inoculated in the same way and under the same conditions. The Havana was affected to approximately the same extent in all the tests and for purposes of comparison was called 100 and all comparisons of others made on that basis.

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[1] Johnson, James, C. M. Slagg and H. F. Murwin. Host plants of Bacterium tabacum. Phytopathology 14: 175–180. 1924.

VARIETIES OF NICOTIANA TABACUM

Almost all of the kinds of tobacco which are cultivated in various parts of the world and come into commerce are forms of the species *N. tabacum*. Comes, the most widely recognized authority on the taxonomy of the genus, divides *N. tabacum* into six primary varieties which have morphological characters sufficiently distinct to warrant a botanist in calling them true varieties.[1] All these varieties however have been crossed and the resulting hybrids crossed again and again until now there are literally hundreds of these creations, many of which have received popular names and are extensively grown locally, *e.g.*, White Burley, Orinoco, Blue Prior, Maryland Broad-Leaf, Zimmer Spanish, Little Dutch, etc. Others are merely the results of long continued selection without crossing, *e.g.*, Havana Seedleaf, Cuban, Pennsylvania Broad-leaf are selections from *N. tabacum* var. *havenensis*. Thus, by these two processes, we have numerous kinds of tobacco and each large tobacco growing section grows one or more kinds which are different from what are grown in other sections. We may designate these as horticultural varieties to distinguish them from the botanical varieties of Comes. The fact that wildfire has been reported from most of the tobacco sections of the United States and that no claims of resistance have been made for any section, leads one to believe that there are probably no resistant varieties among those cultivated in this country. Among the four varieties cultivated in the Connecticut Valley, the writer has never noticed any difference in amount of infection. Varieties from other countries, however, have not been tested, as far as any published records show. The writer was able to obtain from various sources 41 varieties, some of them botanical varieties, but mostly horticultural varieties. All of these were grown simultaneously and inoculated in the same way. As indicated in Table 1, all of them were found to be susceptible to wildfire and most of them did not differ materially from our Connecticut Havana strain in severity of infection. Those which showed the least amount of infection were Maryland, Hester, Bafra, and Sumatra, in the order named. The differences between the others were hardly sufficient to be significant. Since only a single test was made, the four last named varieties are being tested again before any definite conclusions are formed as to their resistance. Johnson, Slagg and Murwin tested five of the above 41 varieties, *viz.*, *angustifolia, atropurpureum, calyciflora, laterrima,* and *chinensis*, and in addition, *macrophylla, trigonophylla,* and *campanulata*. They reported all as uniformly susceptible. (The inclusion of the last named variety under *N. tabacum* is probably an oversight. Dr. Johnson very kindly sent some seed of it to the writer, who found it to be a typical rustica-form—*N. rustica* var. *texana* of Comes.)

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[1] Comes, O. Monographie du genre Nicotiana. Naples. 1899.

TABLE 1.—*Relative susceptibility of species and varieties of Nicotiana in comparison with Havana seed leaf = 100.*

Species and Variety	No. of trials	Average condition compared with Havana 100
N. Tabacum L.		
var. lancefolia (W.) (*N. angustifolia* Ehrh..)	1	80
" fruticosa Hook	1	65
" brasiliensis	1	100
" sanguinea (macrophylla purpurea)	1	80
" chinensis	1	100
" calyciflora	1	100
" atropurpurea (purpurea?)	1	100
" laterrima	1	140
Havana seed leaf	10	100
Cuban seed leaf	10	100
Ct. broadleaf	10	100
Pa. broadleaf	1	100
Sumatra	1	30
Yara	1	100
des Indes Rano de Sumatra	1	100
Zimmer Spanish	1	100
Comstock Spanish	1	100
Small Havana	1	130
Canelle	1	110
Mexican	1	100
White stem Orinoko	1	100
Little Dutch	1	125
Allateban	1	80
White Burley stand up	1	125
White Burley broad leaf	1	100
Blue Prior	1	125
Big Ohio	1	100
Turc aromatic	1	60
de Hongrie Muscatel	1	100
de Hongrie Szamoshati	1	100
Delhi	1	100
grandiflora purpurea	1	100
des Indes Pajacombo	1	100
Persian rose	1	100
Hester	1	10
Espado	1	100
Samsoan	1	75
Obowig long leaf	1	90
Bafra	1	20
Maryland	1	3
Hickory Prior	1	100

TABLE 1.—(*Continued.*)

Species and Variety	No. of trials	Average condition compared with Havana 100
N. rustica L.		
var. humilis	2	0
" asiatica (English tobacco)	2	0
" texana (*N. campanulata*)	2	0
" brasilia (Erbasanta)	1	0
" Iowa	2	0
Brasile lecesse	1	0
Makhorka	1	0
N. alata Lk.		
var. persica	6	0
" grandiflora Com. (*N. affinis*)	3	2
affinis hybrida	1	1
N. acuminata Grah.	2	55
N. attenuata Torr.	1	0
N. Biglovii Wats.	1	40
N. Colossea Andr. (*Lehmannia tomentosa* Spr.)	1	100
N. glutinosa L.	2	75
N. glauca Grah.	1	120
N. Langsdorffii Weinm.	1	95
N. longiflora Cav.	2	35
N. nudicaulis G. Watson	2	0
N. paniculata L.	2	125
N. plumbaginifolia Viv.	2	135
N. quadrivalvis Pursh var. *multivalvis* Gr.	1	85
N. repanda W.	2	0
N. Sanderae (*alta grandiflora* x *Forgetiana*)	2	30
N. suaveolens Lehm.	1	25
N. sylvestris Speg.	1	125
N. wigandioides Englm.	1	100

VARIETIES OF NICOTIANA RUSTICA

The tobacco which was used by the Indians of continental North America before the time of Columbus and which was first introduced into Europe was *N. rustica* L, indigenous to Texas and Mexico. The writer was able to secure seed of seven varieties of this species as given in Table 1. (The last two varieties may not be distinct from some of the others, however.) All the varieties are very rapid, sturdy growers, easily distinguished from any of the varieties of *N. tabacum,* not only by the short yellow flowers and globose pods, but also by the thick blue-green leaves. Eleven sowings were made with the seven varieties and all were thoroughly inoculated just as the other species of Nicotiana, but, in striking contrast to the varieties of *N. tabacum* growing all about, no wildfire appeared on any of them. On the thousands of plants inoculated during two years perhaps a dozen wildfire spots have been found and these developed hardly at all. There was no difference between the seven varieties; all were uniformly so resistant that they could virtually be called immune. Johnson, Slagg and Murwin report this as a susceptible species but do not state what variety was tested.

VARIETIES OF NICOTIANA ALATA

During the summer of 1923, the writer inoculated a greenhouse bed of some thousands of plants of Havana seed-leaf with the purpose of finding whether any individual plant could be found which showed resistance. The bed was inoculated every evening for two months and as fast as any plants showed considerable infection, they were culled out. The result was 100 per cent. of infection on the Havana plants, but nine plants were found growing among the others which were different from the Havana plants and which (with one exception) were immune from wildfire. When they came into bloom they were identified as belonging to the species *N. alata* but not corresponding exactly to any described variety of that species. The fact that most of the pollen was sterile, that only five of the plants ever set seed—and then only a few pods—and that their characters were not entirely uniform, indicated a hybrid origin. They resembled most closely *N. alata* var. *persica*, the famous Persian tobacco, native of South America but grown most extensively in parts of Persia, and used mostly in blending, on account of its superior aroma. The seed of the few pods which matured was sowed and the resultant plants thoroughly inoculated, but they were again found to be immune. This form is called simply *N. alata* in further investigations, since it could not be definitely placed in any described variety.

Another variety of *N. alata*, extensively used as a garden ornamental on account of its very large, showy, white flowers is variety *grandiflora*, commonly sold under the horticultural name of *N. affinis*. Seed of this variety from three different sources was sown and the plants in every case found to be highly resistant, but not quite immune. A purple variety received from Vilmorin under the name of *N. affinis hybrida* also developed barely 1 per cent. of infection. *N. Sanderae*, a hybrid between *N. affinis* and *N. forgetiana*, was tested from two lots of seed from different sources. The first planting developed 60 per cent. infection, the second hardly 1 per cent. Since the parent species, *N. forgetiana*, has not been tested for susceptibility, no explanation of the erratic behavior of the hybrid is offered.

Altogether, the forms of *N. alata* show a very marked resistance to wildfire, amounting almost to immunity.

OTHER SPECIES OF NICOTIANA

Sixteen other species, as named in Table 1, were tested in the same manner as the above. As indicated there, the amount of susceptibility varied from those which were quite immune to others which were much more severely affected than *N. tabacum*. *Nicotiana repanda* and *N. nudicaulis* were particularly prominent for the high degree of resistance exhibited. No spots appeared on either of them. *Nicotiana repanda* is reported by Johnson, Slagg and Murwin as a susceptible species along with eight more of the sixteen listed here.

HYBRIDS BETWEEN RESISTANT AND SUSCEPTIBLE SPECIES

It has long been known that, although in nature there is very little hybridizing in the genus Nicotiana, all the varieties within a species may be crossed at will and that hybrids may be produced even between many of the species. It was hoped that some resistant variety might be found in the genus *N. tabacum* which would be resistant enough to serve as a parent for breeding a resistant desirable strain of Havana, but no such variety has yet been fully demonstrated. In order to watch the behavior of the resistance factor in crossing, it was decided to cross some of the most resistant species with Havana and determine the condition of the hybrids as to wildfire susceptibility by inoculations. *Nicotiana rustica, N. alata, N. nudicaulis, N. repanda* and *N. Sanderae* were selected as representatives of resistant species. (In a later test Sanderae did not prove so resistant.) All efforts to cross *N. rustica* (varieties Iowa, humilis and English) with *N. tabacum* failed, although it is reported that this cross has been made by others.[1] The same lack of success characterized all efforts to cross *N. repanda* and *N. tabacum.*

Nicotiana alata x *N. tabacum.* It was not found possible to make this cross when *N. alata* was used as the female parent, but it was comparatively easy to get seed when *N. tabacum* was the female parent. The vitality of the seed, however, was very low. Not over 1 per cent. of the seed grew, but, after starting, the plants grew vigorously. The hybrids did not vary markedly in characters despite the suspected hybrid condition of the male parent. They were almost perfect intermediates between the parents in stature, size and shape of leaves, habit of growth, color, shape and size of flowers and most, but not all, of the other vegetative characters. The hybrids proved to be entirely resistant like *N. alata i.e.,* resistance seems to be the dominant character. The pollen of the hybrid is sterile and, although fifty or more plants were grown to maturity, not a single pod of seed was matured. The pods drop immediately after the corolla withers. Even when the hybrid flowers were pollinated with pollen from *N. tabacum* no seed was produced. Crosses were also made between *N. alata grandiflora* and *N. tabacum,* but the seed did not germinate.

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[1] East, H. M., and H. K. Hayes. Heterozygosis in evolution and in plant breeding. U. S. Dept. Agri. Bur. Pl. Ind. Bul. 243. 1912.

Nicotiana nudicaulis x *N. tabacum.* The experience with this cross was identical with that of the *N. alata* x *N. tabacum* hybrid in that they could be crossed only when *N. tabacum* was the female parent, in the low germination, in the blending of the characters of the parents, sterility of pollen and lack of seed set. These hybrids were found to be entirely resistant like the male parent.

Nicotiana Sanderae x *N. tabacum* was a repetition of the above results except that the resultant hybrid was slightly susceptible (as the male parent also proved to be in later tests).

There are other possibilities which have not yet been tried but up to the present, the outlook for securing a resistant tobacco by interspecific crossing does not look encouraging on account of the failure of the hybrids to set seed.

SUMMARY

1. None of the 41 horticultural and botanical varieties of *Nicotiana tabacum* has been shown to possess any significant degree of resistance to wildfire.
2. All varieties of *Nicotiana rustica* and *N. alata* which were tried, also *N. repanda, N. nudicaulis* and *N. attenuata* are highly resistant.
3. *Nicotiana acuminata, Biglovii, colossea, glutinosa, glauca, Langsdorffii, longiflora, paniculata, plumbaginifolia, quadravalvis, Sanderae, suaveolens, sylvestris* and *wigandioides* are susceptible but vary in degree of susceptibility from some which are fairly resistant to those which are much more susceptible than *N. Tabacum.*
4. When the resistant species *N. nudicaulis* and *N. alata* are crossed with the susceptible *N. Tabacum* the resultant hybrids are resistant.

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FIELD OBSERVATIONS ON FALSE BLOSSOM OF THE CULTIVATED CRANBERRY

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False blossom is perhaps less understood than any other disease of the cultivated cranberry. It is no exaggeration to state that although published reference to this disease was made in 1908 and the disease has certainly been present, at least in Wisconsin, for many years, there is no agreement as to its cause nor has any practical means of control been discovered.

It may fairly be questioned whether from a strictly scientific point of view it is worth while to publish a discussion of a disease about which so little is known. Practically, however, it is necessary for cranberry growers to deal with this problem and a summary of field observations to date may be of value.

KNOWN DISTRIBUTION

False blossom was first discovered in Wisconsin and is more generally distributed and important in that state than any other. The disease is well established, however, in the cranberry regions of Massachusetts and New Jersey. In Oregon it was found in 1922 on all bogs on which vines from Wisconsin had been planted. False blossom is known to be present on over forty different bogs in Massachusetts and a list of known infections is kept by the staff of the cranberry station at East Wareham. Although no systematic search has been made in New Jersey, the disease is known to occur in various widely separated localities within that state.

VARIETAL RESISTANCE OR SUSCEPTIBILITY

At present there is no widely grown variety of cranberry which is free from false blossom. In 1920, Fracker (1) called attention to the fact that in the course of several years nursery inspection in Wisconsin no false blossom had been found on vines of the Searls variety and that it was rare on McFarlins. The following spring (1920) the writer planted a barrel of Searls vines on a newly scalped area in one of the worst infected bogs in Massachusetts. Within three years many of these vines were badly infected and in 1924 false blossom was present on a large majority of the Searls vines. In 1922 false blossom was found on vines of the Searls variety in Oregon and it has been found on McFarlins in both Massachusetts and New Jersey.

Locally, at least, there is an apparent difference in the susceptibility of different varieties. The Holliston (Mamouth) variety seems to be very generally infected in Plymouth County, Massachusetts. In the same region Howes seem to be more generally infected than Early Blacks. The only New Jersey bog on which Hollistons are known to have been planted is badly infected with false blossom. Here, as in Plymouth County, Massachusetts, Howes are more generally infected than Early Blacks. Native Jerseys are, however, very susceptible. These relations may not hold in other regions.

The statements made in this paper as to the occurrence of false blossom on different eastern varieties are not based merely on finding the disease in a section planted to a given variety, but in each case typical berries have been found on vines affected with false blossom. In almost any bog in which the infection is not too severe it is possible with sufficient care to separate from the mass of intertwined vines single plants, one portion of which is apparently normal, or at least bears normal berries, and another portion of which is obviously affected with false blossom. This observation has been repeated many times and may be readily verified by any one who will take time to separate out the vines. The vines may be more easily separated of course on unsanded bogs. In no case has the writer relied solely on his own judgment in determining the variety but has referred the specimen for identification to some one whose familiarity with the variety would place the determination beyond question, Dr. Franklin in Massachusetts, Miss White or Mr. Scammell in New Jersey, and Mr. Lewis in Wisconsin.

THE SPREAD OF THE DISEASE TO NEW VINES

In the absence of definite proof of the nature of the disease, opinion as to its spread must be based on field observations. In 1914 Shear and Franklin made a survey of false blossom conditions in the vicinity of North Carver, Massachusetts, where they found the disease present on five bogs. Their observations, which were reported separately (1, 2), are in full agreement that in all cases the vines worst infected with false blossom were Wisconsin vines which had been planted on small areas, and that there was very little infection on Massachusetts varieties. In discussing the apparent spread to eastern varieties, Franklin (p. 100) uses the terms "was found to some extent" and "to a slight extent," while Shear says (p. 3), "In both Massachusetts and New Jersey a few scattered vines showing the disease have been found in plantings of eastern varieties in the same bogs." The New Jersey bog here referred to is one on which a small plot of Wisconsin vines had been planted about 1909, and on which false blossom was first noticed in 1915.

The reports of these careful and competent observers can leave no doubt that in 1914 the infection of Eastern varieties on these six bogs was not severe. The Wisconsin vines were, however, removed from all five of the Massachusetts bogs within the next year.

In 1924, ten years after the observations reported by Shear and Franklin, the following condition exists: False blossom is present on undoubted eastern varieties on all five bogs at North Carver, Massachusetts. On the most severely infected of the bogs false blossom is found to some extent over practically its entire area (eighteen acres), while several sections have become so badly infected as to become worthless and have been torn out and replanted. On the other bogs false blossom is present to an injurious extent over areas varying from one-half acre to several acres, a condition which contrasts strikingly with the "few scattered vines" of the 1914 report. The New Jersey bog referred to in Shear's report was visited in 1924 by Miss Elizabeth White and the writer. False blossom is now present on Centennials over practically the entire bog and is readily found at a distance of one hundred yards or more from the original planting of Wisconsin vines.

Such observations suggest the possibility that false blossom is an infectious disease and has spread to the eastern vines from the diseased vines which were planted near them. If, as has been suggested, false blossom is "a physiological disturbance due to unfavorable cultural conditions," the conditions on all six of these bogs must have been so unfavorable as to induce the spread of the disease in varying amounts. This is, of course, possible.

It does not seem at all probable, however, that unfavorable cultural conditions can account for the following instance. In 1915, after the general survey of the false blossom situation in Massachusetts already mentioned, Dr. Franklin planted three sods of Wisconsin vines affected with false blossom on one of the sections (Number 13) of Howes in the State Experiment bog at East Wareham, a careful survey of the state bog having failed to show any false blossom. At that time it was generally believed that false blossom was not infectious and that diseased plants would recover under favorable conditions. The test was made to determine whether, under the cultural conditions found at the state bog, the affected plants would recover. The transplanted false blossom vines did not recover, but after a few years died out and the space was filled with Howes vines. At the present time (1924), however, there is some false blossom on the Howes near where the diseased sods were set and a locally severe infection of Howes on Section 7 across the main ditch. A natural inference would be that the false blossom had spread from the introduced infected vines to the Howes.

Related to the question of spread to new vines is the question of the distribution of the disease by the setting of diseased vines. As already noted, false blossom was found in Oregon on all bogs which had been planted with Wisconsin vines, many of which were presumably infected with false blossom. Thus far, indeed, it has been found in Oregon only on Wisconsin varieties. Franklin and Shear both call attention to the fact that false blossom in Massachusetts and New Jersey was first found on bogs where infected Wisconsin vines had been planted.

In pointing out these instances in which false blossom has apparently been introduced on eastern bogs by the planting of diseased vines from Wisconsin, it is not intended to convey the impression that such infections on eastern bogs can always be traced to Wisconsin vines. There are numerous cases in which false blossom occurs on eastern bogs where no Wisconsin vines have been set. On the other hand, the available evidence indicates that on the seven eastern bogs just referred to the first observed infection was on Wisconsin vines, and that a common means by which false blossom is introduced into new localities is by the shipment of vines already infected.

While it is certainly true that false blossom now occurs on various bogs in which no infected vines are known to have been set, it is on the contrary often possible to trace the source of at least part of the vines to some bog known to be infected. These observations suggest that it is extremely unwise to plant vines from a bog infected with false blossom. Fracker (1) sums up the situation in Wisconsin as follows:

> Even though the cause may be a physiological one, plants from infected beds continue to develop false blossoms after being transferred to new locations. The loss usually then becomes greater from year to year and total crop failure sometimes results, followed by the abandonment of the bogs. Under these circumstances the nursery inspection office must adopt the same policy in providing for cases of false blossom as if the disease were provéd to be of an infectious nature.

The present writer's observations also indicate that this is the only safe course. No case is known to the writer in which a bog once known to contain false blossom plants has become free from the disease.

THE "RECOVERY" OF VINES AFFECTED WITH FALSE BLOSSOM

This raises the question of the recovery of vines affected with false blossom. Thus far all infected vines marked for observation have either continued diseased or have died. As it is not possible to keep a very large number marked, this does not disprove the possibility of their recovery. On the other hand, it is readily observable that under conditions favorable for the growth of healthy vines part of the diseased vines die out and healthy ones crowd in and take their places. This condition, the writer believes, accounts for many of the reports of "recovery" of infected vines.

RELATION OF THE DISEASE TO CULTURE CONDITIONS

In earlier publications on false blossom the disease has generally been attributed to unfavorable cultural conditions, particularly to lack of drainage or to extreme drought. Recently Scammell (1) in discussing false blossom in New Jersey said:

> False blossom is not a serious trouble with us but I have found it occurring on numbers of bogs in New Jersey, attacking such varieties as Howes, Early Black, Centennial and Jersey. It appears on our mud bottoms and our savannas, where drainage is good and where drainage is poor.

On the other hand, Franklin (5) makes the following observations:

> We have come to regard a severe attack of this disease as a mark of poor cultural conditions. Anything tending to weaken the vines seems to give it a chance. . . . We have been finding it increasingly on the bogs the last few years and we know it has increased greatly in some cases. . . . Girdler injury seems to make the vines especially susceptible to the disease.

The very week in which this part of Dr. Franklin's report was published in the Wareham Courier, Mr. Beckwith of the New Jersey Experiment Station stated to the writer that according to his own observations false blossom was most common on bogs weakened by attacks of the cranberry girdler (*Crambus hortuellus* Hübn).

No contradiction is involved in these statements, which agree in general with the writer's own observations. That is, *severe* attacks of this disease seem to be associated, at least in Massachusetts and New Jersey, as indicated by Franklin and Beckwith, with poor cultural conditions or unusual abundance of insect pests. On the other hand, the disease certainly seems to be able to maintain itself, as pointed out by Scammell, under a variety of conditions and is certainly known to occur at present on several bogs in Massachusetts on which cultural conditions, as indicated by yield and the keeping quality of the crop, are far above the average.

Several years ago it was stated on the authority of a western cranberry grower that plants from Wisconsin showing the disease had entirely (Shear, p. 5) recovered from the disease when grown on the Pacific Coast. When, however, the writer visited this region in 1922 the disease was found to some extent on every bog on which Wisconsin vines had been planted. It seems, however, to be of slight importance in this region.

It appears then that while excellent cultural conditions and the control of insect pests appear to check the rate of spread of false blossom and may even reduce the amount of disease by preventing its spread and favoring the continued growth of healthy vines to take the place of those killed by the disease, there is no indication at present that the disease may be eliminated by improving cultural conditions.

SUGGESTIONS FOR CONTROL

Whether false blossom is finally proved to be an infectious disease or not, it seems wiser to treat it as such in the field.

In case of a scattered infection it is highly desirable to improve cultural conditions in the hope that the disease may be reduced by the death of the diseased vines and the continued growth of the healthy ones.

At least one grower gets good results on a bog having a scattered infection by systematically pulling out vines infected by false blossom when weeding the bog.

On one Massachusetts bog which was in a serious condition from false blossom, Scammell's suggestion has been followed and a chemical weed killer has been used in an effort to kill all the cranberry vines preparatory to replanting with healthy ones. This drastic treatment, however, is still in the experimental stage and its general adoption is hardly to be recommended at present.

The most important precaution is to avoid planting vines from bogs known to be infected with false blossom.

It will be evident from the observations just summarized that we are still very much in the dark in our efforts to combat false blossom and it is a source of much satisfaction to those of us who are interested in the practical control of cranberry diseases that the study of false blossom has been taken up by an investigator who has had marked success in dealing with insect-borne diseases of the mosaic type.

SUMMARY

Field observations indicate that false blossom has spread on certain bogs in Massachusetts and New Jersey since its discovery there ten years ago.

Eastern varieties are now known to be infected.

The disease is able to maintain itself under very good cultural conditions.

It is suggested that until the cause of the disease is definitely established it will be wise to treat it as infectious, and carefully avoid the sale or planting of diseased vines.

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FUSARIUM ROT OF THE PEACH

A. G. Plakidas

In a recent paper, J. W. Roberts (1) described a bud rot of the peach, reported from Georgia, and caused by a species of Fusarium. The purpose of the present paper is to report: (1) The occurrence of Fusarium fruit rot of the peach in California, and (2) the development of a bud-rot, similar to that described by Roberts, on peach and apricot by artificial inoculation in the laboratory with pure cultures of three forms of Fusarium isolated from decaying peach fruit. The writer believes that this report will be of interest from the viewpoint of the geographical distribution of this type of rot.

As far as the writer knows, nothing has been published concerning Fusarium rot of the peach or of other stone fruits. In August, 1920, Prof. W. L. Howard sent from Mountain View, California, two specimens of peach fruit showing a Fusarium rot. Professor Elizabeth H. Smith, who examined these specimens, gives the following description of the rot: "This rot has never been seen previously, and it is interesting as Fusarium has never been known to take hold of the peach pulp in this way. The kernel and shell are badly damaged from the physiological gumming and splitting which has given entrance to the fungus. Two affected fruits were submitted, both showing the typical effect. A large area, including about one-half of the fruit, shows a dry rot, apparently starting at the tip and extending to the stone throughout the rotted area, the surface being covered with a thick felty layer of the mycelial growth. This is a pale pink in color, distinctly zonate and with a dense layer of spores at the base of each zone of several shades deeper salmon pink color."[1]

B. A. Rudolph, of the Mountain View Experiment Station, has observed the occurrence of Fusarium rot of peaches in the Santa Clara Valley, and has collected and examined specimens. Acknowledgment is made to Mr. Rudolph for the following information: "The fruits of both Phillips and Tuscan clings are affected, and possibly many others. The fruit is attacked just about the time it ripens. Affected fruits usually drop to the ground, but I have seen fruits in which about one-quarter of the flesh was involved still hanging on the trees. The rot I believe to be relatively a slow one. Certainly, affected fruit is not destroyed with the rapidity of, say, brown rot. Thus far the disease has been of no economic importance. However, it should be recognized."

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[1] The author is indebted to Prof. E. H. Smith for the use of her records and of the plates of the photographs shown on Fig. 4.

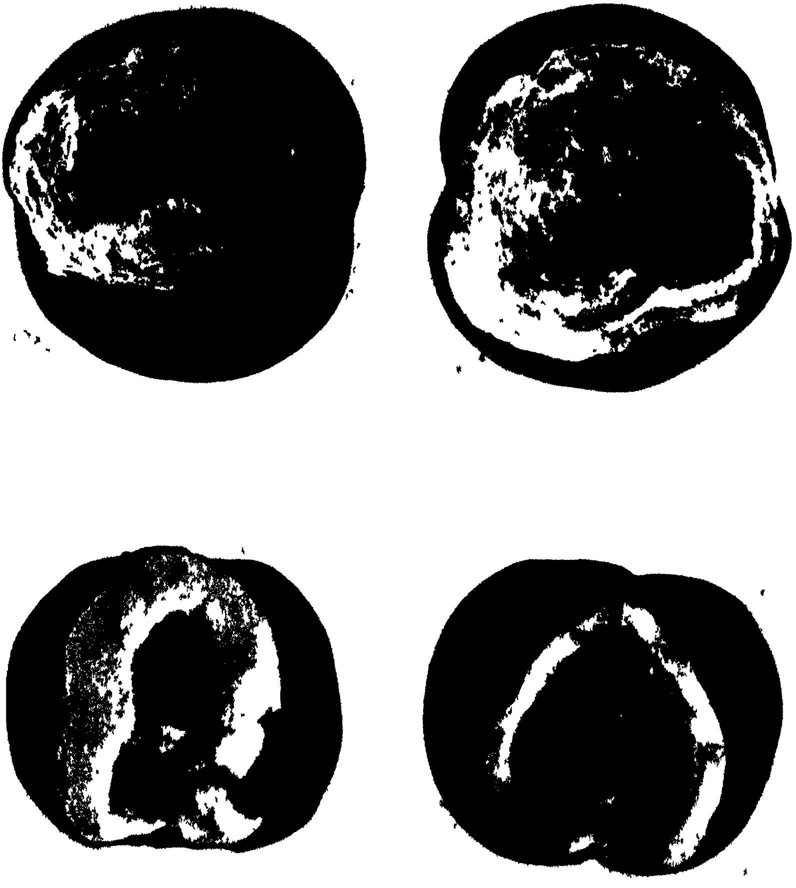

FIG. 4. A. Peach fruit showing Fusarium rot. From specimens sent in by Prof. Howard from Mountain View, Santa Clara Valley, in Aug., 1920. B. Specimen from the same location, collected by B. A. Rudolph, in 1922.

Early in September, 1923, the writer received a number of specimens of decaying peaches from two localities in Sutter County, Sacramento Valley, Yuba City and Nicolaus. All of these specimens were of the Phillips cling variety. Among these specimens, some were found that showed the characteristic Fusarium rot (Fig. 5 A). It is worth noting here that out of 48 specimens comprising the Yuba City lot, 4 showed Fusarium rot, while 3 more exhibiting the same kind of rot were found among the 56 specimens of the Nicolaus lot. Since the specimens were picked at random from different parts of the orchard, the fact that about 7 per cent showed Fusarium rot illustrates the prevalence of this type of rot in this region that season.

Three distinctly different forms of Fusarium were isolated from these specimens. Pure cultures of these were secured by single spore isolations.

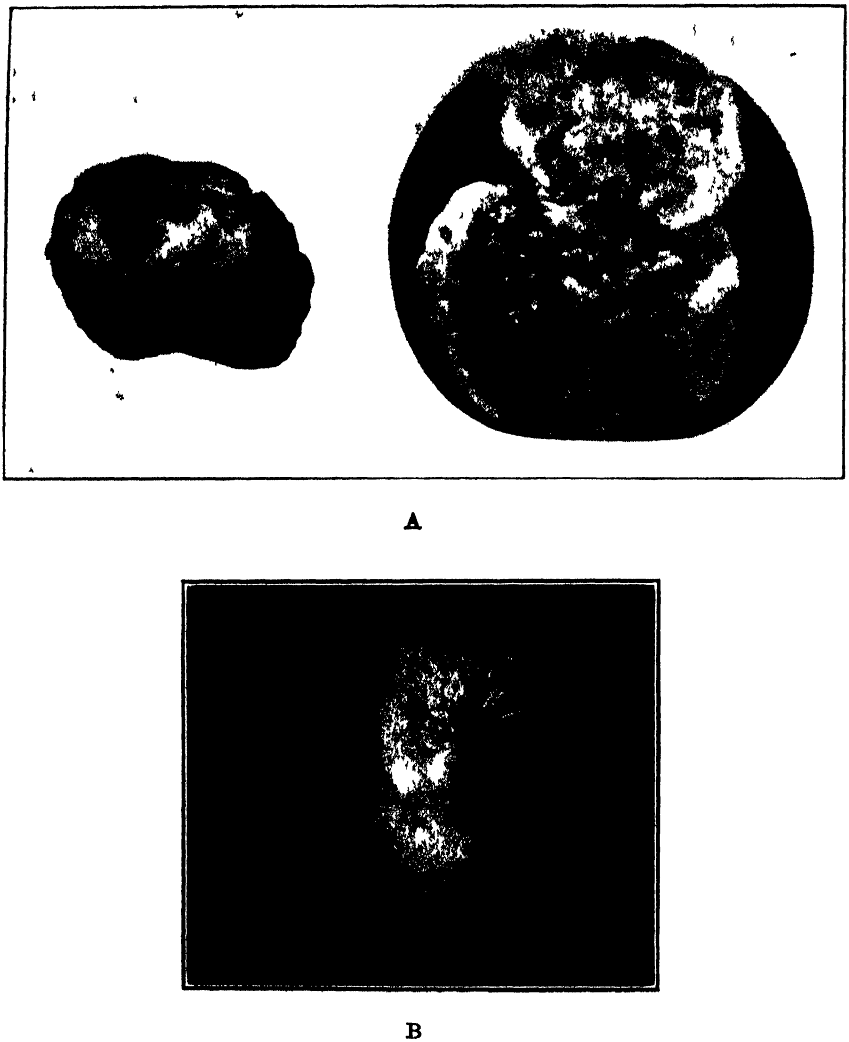

FIG. 5. A. Peach fruit showing Fusarium rot. Specimen collected from Yuba City, Sacramento Valley, Sept., 1923. B. Peach rot produced by artificial inoculation in the laboratory with pure culture of *F.*-(X_4) *pirinum* (?)

Dr. Sherbakoff identified these forms, more or less definitly,[2] as *Fusarium asclerotium* (Sherb.) Wr., *Fusarium solani* (Mart. p. part.) App. et Wr., and *Fusarium pirinum,* respectively. Therefore, it is not considered necessary in this paper to give a detailed description of these fungi.

EXPERIMENTATION

1. *Fruit inoculations:* Inoculations were made with pure cultures of these forms on fruit; peaches (Salway), oranges (Washington Navel), apples (Oregon Pippin), and lemons, in moist chambers in the laboratory. Several such inoculations were made from September to December, 1923. Both mycelium and spore inoculations were tried, puncturing and without puncturing the skin of the fruit. The fungi failed to grow on the apples and lemons, but typical Fusarium rot was produced on the peaches and oranges in every instance. (See B, Fig. 5) It must be stated, however, that the rate of decay, under laboratory conditions, was slow in every case.

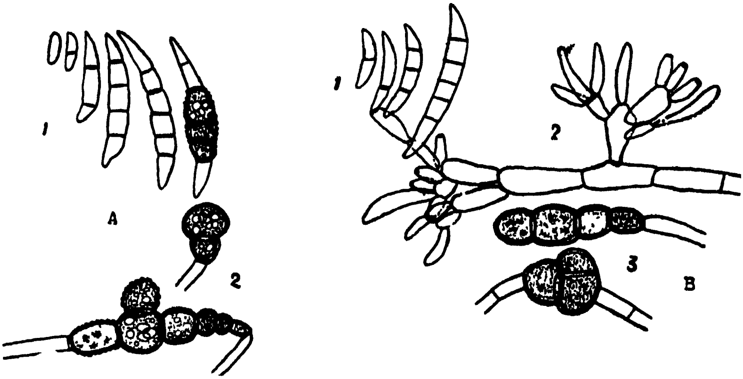

FIG. 1. Conidia, chlamydospores, and sporophores of $F\text{-}X_1$ (*F. asclerotium* (Sherb.) Wr. as suggested by Sherbakoff)

A. From potato plug, culture, 50 days old. 1. Conidia × 1000. 2. Chlamydospores × 1000. B. From nutrient agar, culture, 7 days old. 1. Conidia × 1000. 2. Conidiophore × 1000. 3. Chlamydospores × 1000.

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[2] This is Dr. Sherbakoff's statement regarding the identification of these Fusarium forms: " . . . please permit me to say that, from my examination of your Fusarium X-1, I believe the form could safely be put into the species *Fusarium oxysporum* var. *asclerotium,* as the name is given in my 'Fusaria of Potatoes,' Cornell Memoir No. 6, or as renamed by Dr. Wollenweber, *F. asclerotium* (Sherb.) Wr.

"Your fungus No. X-3 is probably identical with *F. solani* (Mart. p. part.) App. et Wr.

"Your fungus X-4 corresponds very much with *F. pirinum* Fries. . . , in general it resembles very much, and probably is identical with, *F. biforme,* the latter fungus to be considered a synonym of *F. pirinum.* This matter was agreed to by Dr. Wollenweber and myself."

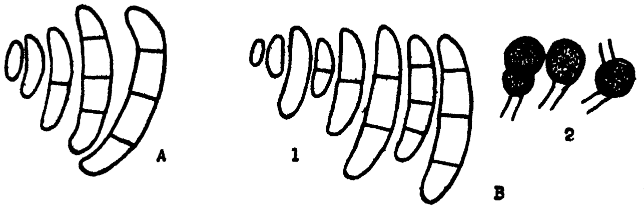

FIG. 2. Conidia and chlamydospores of F–X_3 (Probably identical with *F. solani* (Mart. p. part.) App. et Wr., according to Sherbakoff)

A. Conidia from nutrient agar, culture 7 days old. × 1000. B. From potato plug culture 55 days old. 1. Conidia × 1000. 2. Chlamydospores × 1000.

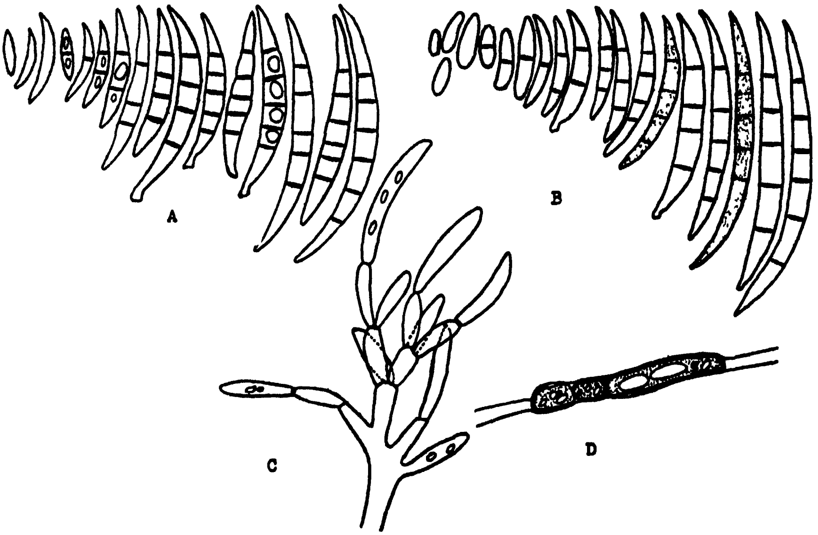

FIG. 3. Conidia, chlamydospore and conidiophore of F–X_4. (Probably *F. pirinum*, Fries)

A. Conidia of different shapes and sizes from nutrient agar, culture 13 days old. × 1000. B. Conidia from potato plug culture, 72 days old. × 1000. C. Conidiophore from potato plug culture, 13 days old. × 1000. D. Pseudo-chlamydospore; from potato plug culture, 72 days old. × 1000.

F. pirinum was the most active of the three, but even this required from 20 to 30 days entirely to rot the inoculated fruit. It was not found possible to try fruit inoculations in the field.

2. *Bud inoculations:* Peach (Elberta) and apricot (variety?) twigs were placed under bell jars, the cut ends of the twigs being immersed in water in tumblers, in the laboratory. One set of twigs was sprayed with a spore suspension in sterile water from *F. asclerotium*, another from *F. solani*, a

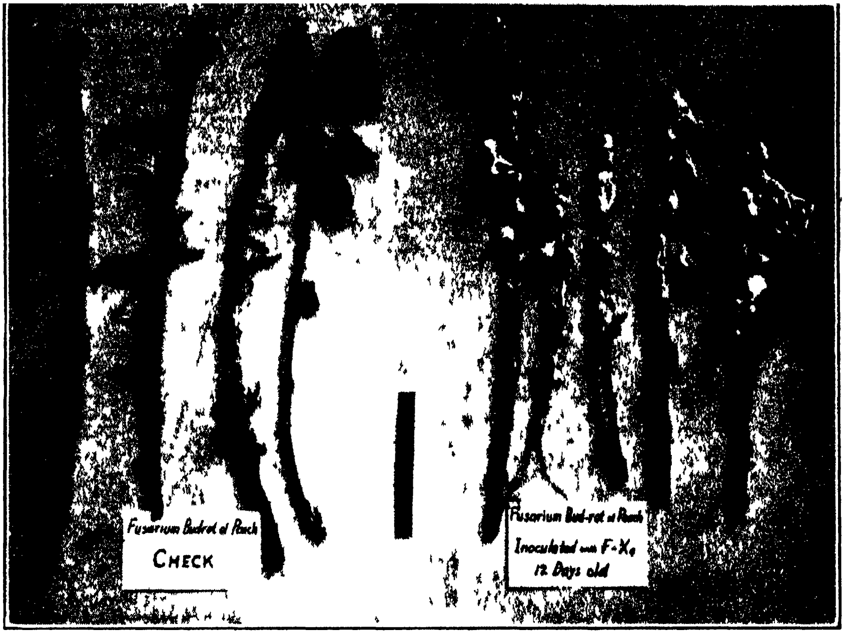

FIG. 6. Bud-rot on peach and apricot twigs, produced by artificial inoculation with spore suspension from a pure culture of *F. pirinum* (?) under a bell jar in the laboratory. The fourth twig in the check, and the fourth and fifth in the inoculated row are apricot; the rest are peach.

third from *F. pirinum,* while a fourth set was sprayed with sterile water to be used as check. The spraying was done by means of an atomizer. This experiment was started on March 14, 1924. Most of the buds, both leaf and flower, at this time were open. The results of this experiment are summarized in the table below:

TABLE 1.—*Relative effect of three species of Fusarium on inoculated peach and apricot twigs, in 10 days*

Organism	Percent of buds killed		Condition of twigs
	Apricot	Peach	
F. asclerotium	50	50	Shriveled
F. solani	100	77	Badly shriveled; dying out
F. pirinum	100	100	Dead
Check	0	0	Fresh

The *F. pirinum* set showed the most conspicuous effects. There was 100 per cent. killing of buds on both the apricot and the peach twigs. Profuse white, cottony, mycelial growth covered the blighted twigs. (See Fig. 6.) The killed buds were dark brown to black in color. At first they were somewhat water-soaked, but soon became dry.

The specific Fusaria were re-isolated in pure cultures, in every case, by plating aseptically some of the inside tissue of the killed buds.

Similar inoculations were made in the garden of the laboratory on twigs of growing peach and apricot trees. This experiment was started at the same time as that in the laboratory, and it was repeated. The inoculations failed to take. This result should not be taken as conclusive, however, especially in view of the fact that it was an unusually dry season for Berkeley at the time that the experiment was carried out. Prof. E. H. Smith told the writer that she has often isolated Fusaria from blighted twigs that are sent to the Station for examination, and that she has considered them as secondary saprophytic organisms. It is possible that, under favorable moisture conditions, like those that prevail in the California valleys during the winter and early spring, some of the killing may be due, partly at least, to Fusaria.

SUMMARY

1. The occurrence of Fusarium rot of the peach fruit in California is reported.

2. Three forms of Fusarium were isolated from specimens of decaying peaches sent in from Sutter County, California. According to Sherbakoff, these three forms correspond to *F. asclerotium* (Sherb.) Wr., *F. solani* (Mart. p. part.) App. et. Wr., and *F. pirinum* Fries, respectively.

3. Inoculations with pure cultures of these three forms produced rot on fruit (peaches and oranges) and on peach and apricot buds under laboratory conditions, but failed to produce bud-rot on growing peach and apricot trees in the field under Berkeley, spring conditions.

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DIVISION OF PLANT PATHOLOGY

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THE CITRUS SCAB FUNGUS

Anna E. Jenkins[1]

With Four Figures in the Text

The disease of citrus commonly known as scab is widely distributed geographically, and in Southeastern United States it is regarded as one of the most important diseases of this host (12, 18). It has also been reported recently on avocado by Stevens (13). While many of the etiological aspects of the disease are known, the systematic position of the pathogene has remained doubtful.

Scribner (12) was of the opinion that the Cladosporium which he found growing on the surface of the old lesions produced the disease. Swingle and Webber (15) likewise attributed the disease to a Cladosporium of which they described the conidiophores and spores and recorded their measurements. Upon their description, which characterizes a true Cladosporium as typified by *Cladosporium herbarum,* Massee (10) based his diagnosis for *Cladosporium citri.*

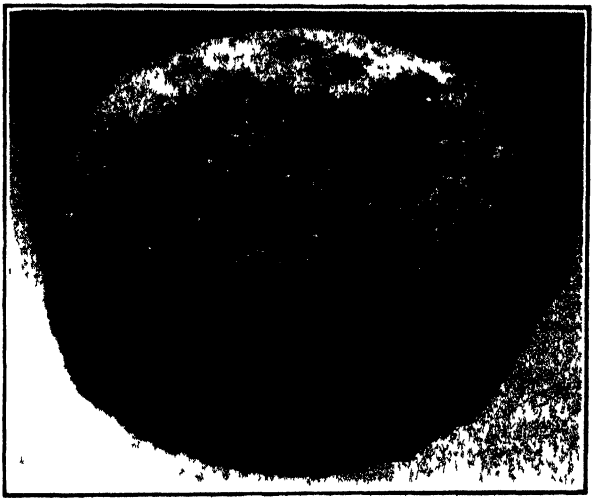

Fig. 1. Citrus scab on mature lemon. Natural size.

The name *Cladosporium citri* Massee, therefore, applies to the fungus observed by Swingle and Webber on the old lesions. Accompanying Scribner's specimens which are now in the Pathological Collections of the Bureau of Plant Industry, is a note by Ellis, dated June 4, 1886, in which he wrote, "I do not think the *Cladosporium* belongs especially to the warts. The Cladosporii grow on all diseased parts." Grossenbacher (9) isolated a Cladosporium from the surface of old scab lesions with which he was unable to reproduce the disease. The writer has also isolated a Cladosporium-like fungus from the lesions; it may be that those referred to in the literature are of this type.

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[1] Paper presented at the annual meeting of the Botanical Society of America, Mycological Section, Washington, D. C., December 29, 1924.

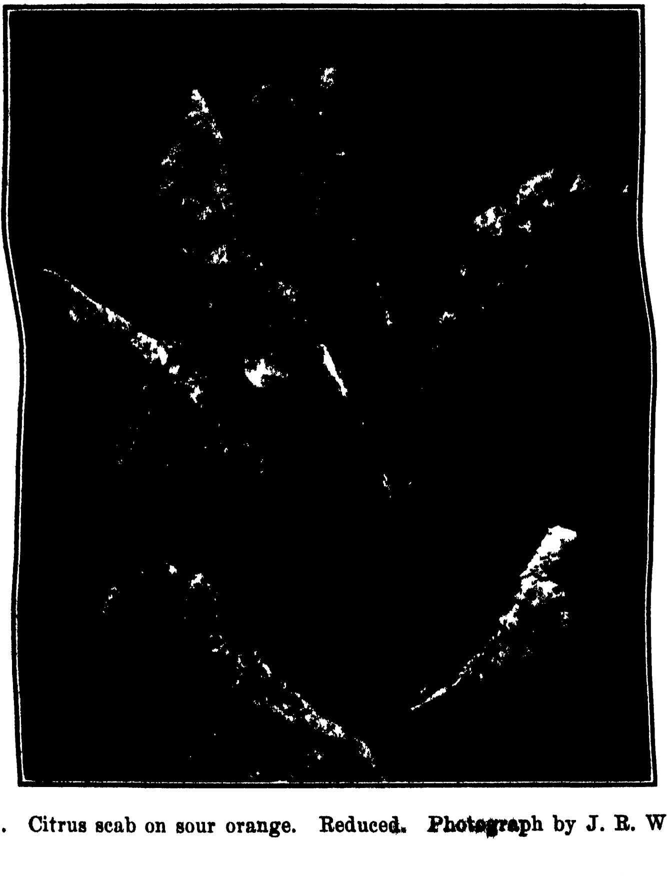

FIG. 2. Citrus scab on sour orange. Reduced. Photograph by J. R. Winston.

When Fawcett isolated the actual pathogene in 1906 (5), he reported it as *Cladosporium citri* Massee. Up to the present time this name has been almost universally used in referring to the citrus-scab organism. But the name as stated above refers to a Cladosporium which is evidently distinct from the fungus studied by Fawcett. In most of Fawcett's publications he states that the pathogene is not typical of the genus Cladosporium and it is apparent that he realized it had no relation to the fungus found on the older lesions (6, 7, 8).

For the purpose of the present study, a single-spore strain of the citrus-scab organism was obtained from a culture isolated by Mr. H. E. Stevens in 1916. Greenhouse and field inoculations[2] of citrus leaves with this single-spore strain have reproduced the disease. Subcultures from this on various media under similar conditions were compared simultaneously with pure cultures of *Sphaceloma ampelinum* DeBary, isolated from typical anthracnose lesions on grape, and with subcultures of *Plectodiscella veneta* (Sacc.) Burkholder, the raspberry anthracnose fungus, grown from a culture contributed by Dr. L. K. Jones. Although the work is still in progress and has been confined largely to observations of growth in artificial media, the results indicate that these three organisms possess certain peculiar morphological and cultural characters in common, suggesting a close relationship. It is to be noted that as early as 1915 Shear's (14) attention was attracted to the similarity in culture of the grape and raspberry anthracnose fungi and at that time he suggested the possibility of their relationship. The parallel cultures show that the citrus fungus resembles *Sphaceloma ampelinum* more closely than *Gloeosporium venetum*, the imperfect stage of *Plectodiscella veneta*. Nevertheless the cultures appear to show that all three are distinct species.

The perfect stage of the raspberry anthracnose fungus is known (1, 2, 3). For the grape fungus for which DeBary (4) created the form genus *Sphaceloma*, Viala and Pacottet (16, 17) report extreme polymorphism, including a budding or yeast-like form which developed asci and ascospores. This ascigerous stage is very different from Plectodiscella, and as no other authors have reported it, a repetition of this work is desirable. As no perfect stage has been observed for the citrus-scab organism, it would seem best to classify it for the present under the hitherto monotypic form genus *Sphaceloma*. Since no technical name appears to have been given for the fungus, the specific name **fawcetti** is proposed. The description follows:

Sphaceloma fawcettii n. sp. (*Cladosporium citri* Fawcett, not Massee). Acerculi solitary or confluent, subcircular, chiefly less than 1 mm. in diameter, on leaves intra-epidermal, becoming erumpent, pseudoparenchymatous at base, may also extend to the underlying host tissue, in this region mainly plectenchymatous; conidiophores arising perpendicularly from surface of stroma, standing close together, cylindrical, apex sharp pointed, blunt apiculate or obtuse, 1 to 3-celled, hyaline but may become dusky, principally 12 — 22 x 3 — 4 μ; conidia acrogenous, also pleurogenous (observed in culture), usually developed singly, often in succession from the same conidiophore (observed in culture), oblong-ellipsoid slightly reniform or ovid, ranging from 5 — 10 x 2 — 5 μ

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[2] The field inoculations were made at Orlando, Florida, October, 1924, by J. R. Winston, Bureau of Plant Industry.

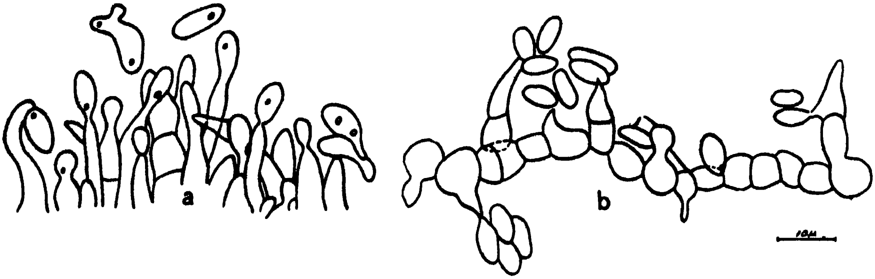

FIG. 3. *Sphaceloma fawcettii* from culture. *a*. Conidiophores and conidia developed on surface of stromatic growth on potato dextrose agar medium. *b*. Formation of conidiophores and conidia from mycelial strand.

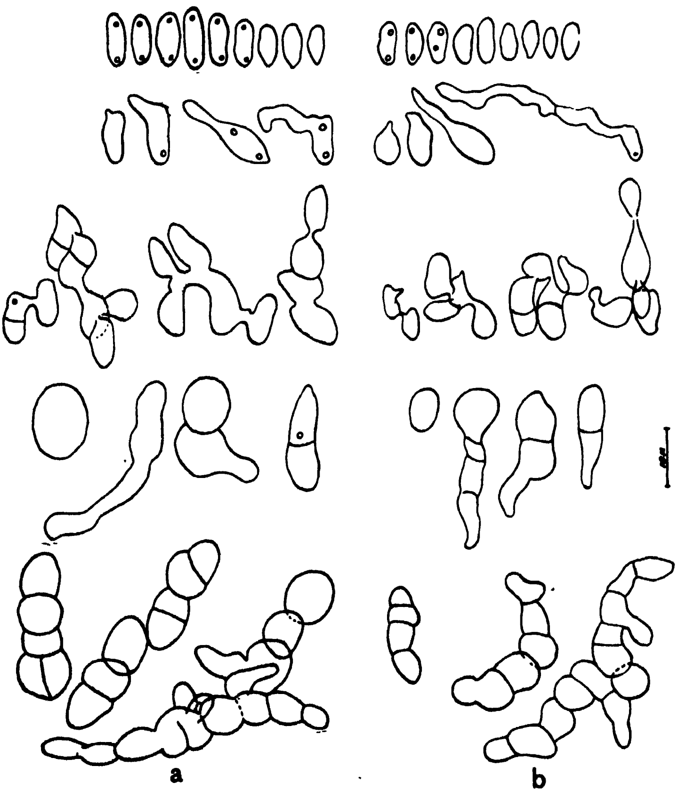

FIG. 4. Drawings to show relative dimensions and similarity of various germinated spores of the citrus scab fungus and the grape anthracnose fungus. *a*. *Sphaceloma fawcettii*. *b*. *S. ampelinum*. Developed in culture.

usually 6 — 8.5 x 2.5 — 3.5 μ, typically bi-guttulate one oil drop at each end, continuous, hyaline, sometimes becoming elongated or swollen and 1-septate, and dusky.

Distribution: On *Citrus aurantium* Linn., *C. grandis* (L.) Osbeck, *C. limonia Osbeck,*[8] producing rough, corky, wart-like projections commonly known as 'scab,' on twigs, leaves and fruits; lesions at first more or less translucent, green or tan, frequently becoming pink to brown at center, on fruit often becoming purplish. Florida, U. S. A. The disease known as scab has been reported from various citrus-growing regions of the world upon other hosts than those listed above.

Specimens examined:

On *Citrus aurantium;* Orlando, Fla., 1924, J. R. Winston, P. C. 9751 (Type); Ocala, Fla., 1886, P. C. 9749; Lake City, Fla., 1906, P. H. Rolfs and H. S. Fawcett, (F. Col. 2316), P. C. 9754; Bartow, Fla., 1900, H. H. Hume, P. C. 9755.

On *Citrus grandis;* Orlando, Fla., 1924, J. R. Winston, P. C. 9752.

On *Citrus limonia;* (artificial inoculation in greenhouse), Arlington Experiment Farm, Rosslyn, Va., 1924, A. E. Jenkins, P. C. 9753.

These specimens, as indicated by their accession numbers (P. C. No.), are to be found in the Pathological Collections, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

Since it appears that the citrus-scab fungus is closely related to the anthracnoses of grape and raspberry, a correlation of the data on these three diseases is possible. Such a correlation might be of mutual aid in a further interpretation of the information in the literature on these three diseases and their causal organisms.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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[8] The plants on which this fungus has been observed in Florida are listed in the paper entitled, "Susceptibility of some Rutaceous Plants and of Avocado Varieties to Attack by the Citrus Scab Fungus," by J. R. Winston, J. J. Bowman, and W. J. Bach, in Journ. Agr. Res. (In press.)

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EFFECT OF SOIL TREATMENT WITH SULPHUR UPON CROWN GALL IN NURSERY APPLE TREES

C. D. Sherbakoff

With Three Figures in the Text

Crown gall of apple in the nurseries of Tennessee and in the other southern states is recognized as a serious trouble. About three years ago the writer carried out an experiment in cooperation with two nurseries at Winchester, Tennessee, on the control of this disease by means of thorough sanitation. The scions and roots were thoroughly disinfected by dipping for 20 minutes in a 1:1000 solution of corrosive sublimate, after presoaking overnight, and then thorough rinsing in clean water. The grafts were made from the scions and roots over disinfected benches. The grafting knives and men's hands were also disinfected from time to time, and the grafts were stored in new boxes with new, clean sand. The grafts were of Early Harvest variety. The outcome showed[1] that in spite of these sanitary measures a very high percentage of the trees developed crown gall.

One of the cooperating nurserymen treated in the same way some of his grafts of the "Horse" and Yates varieties and reported, as a result of the treatment, considerably less crown gall in the Yates than he usually had in this variety.

The results of the experiment with Early Harvest alone indicate the ineffectiveness, or at least not a sufficient effectiveness, of the sanitary measures by themselves, and the conclusion is reached that the bacteria are present either in the scion (or root) or in the soil.

During 1922 and 1923, in cooperation with Mr. J. A. McClintock, the work was directed primarily along two lines: one to determine whether the infection in Early Harvest is carried internally, and whether there are some specimens of the variety that are free from such internal infection; and the other to test again the effect of the sanitary measures on crown gall development in Early Harvest as well as in several other varieties of apple trees. The work was done on the grounds of the Tennessee Experiment Station, at Knoxville, with a number of varieties of scion wood obtained from several different sources. Most of the grafts were made under sanitary conditions similar to those employed during the preceding year, as previously outlined. The writer with Mr. McClintock expects to publish in due time a detailed account of these experiments. For the present it is sufficient to say that out of the total of 2,320 one-year-old nursery trees of the Early Harvest variety grown, 413, or 18 per cent, showed true crown gall.

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[1] The data were collected by J. A. McClintock.

The above figures show about the same result as that of the preceding season, namely, an ineffectiveness of the sanitary measures. Judging from the fact that nearly all of the Early Harvest from different sources showed considerable crown gall, one is forced to conclude that the bacteria were probably present in the soil. Therefore it was decided to conduct a preliminary experiment on control of crown gall by a soil treatment.

The writer's observations and those of the nurserymen agree in that crown gall is worst under conditions of an abundant humus supply in the soil, especially where there is an application of barnyard manure, and where the soil is well supplied with lime—conditions similar to those which favor the common scab of Irish potatoes. The similarity goes farther. Both pathogenes—that causing the scab and the other causing crown gall—are intolerant of an acid reaction of the medium. The writer's previous experimental work with sulphur treatment of soil against the scab (See Cornell Bull. 350, Aug., 1914) suggested the same treatment for the control of crown gall.

A corner of the field on the grounds of the Experiment Station at Knoxville where infected apple trees were produced during the preceding season was selected for the experiment. Fifteen rows were laid off 3½ feet apart and about 100 feet long. Eight rows (every odd row) were left untreated as checks and seven rows (every even row) were treated with sulphur at the rate of 4.8 pounds per row, about 600 pounds per acre. The grade of sulphur was that which is known on the market as "inoculated sulphur." It was applied by hand evenly over a shallow furrow, about 3 or 4 inches wide, made with a hand plow. The furrow was covered with an attachment to the hand plow; then opened with the plow, then covered again with the attachment. This was done in order to have the sulphur well mixed with soil throughout a narrow strip where the grafts were to be set. The 600 pounds per acre was used because it appeared to be a sufficient amount to be effective on the particular piece of land and not too heavy to be injurious to the young apple trees. The sulphur was applied a week before the grafts were planted.

For this experiment, bench grafts were made on March 20 and 26, 1924, from root cuttings of commercial apple seedlings and from scion wood cut off from one-year-old nursery trees affected with well-developed crown gall at the union or on the scion wood. In making the grafts no sanitary precautions were taken and the scions and roots were not disinfected. The grafts were wrapped in damp sacks and stored in a cellar until April 7, when they were set in the field, in the sulphur-treated and untreated rows.

There were planted 1,575 grafts of Early Harvest, 270 of Red Delicious, 150 of Rome Beauty, and 111 of "Horse," each variety in a separate block across all of the fifteen rows and with as nearly the same number per row as possible. Owing to the poor condition of the root cuttings used in making the grafts, only about one-third of them rooted and produced normal trees.

The trees infected with well-developed crown gall, from which most of the scion wood was cut off for the making of the grafts for this experiment, were also planted in the sulphur-treated and untreated rows, at one end of the plot, nineteen trees per row.

From the beginning to the end of the season no difference between the trees grown in sulphur-treated and untreated rows could be detected, either in the stand of the trees or in their size. On November 3, 4 and 5, the trees were lifted and examined for crown gall.

The occurrence of crown gall infection on the nursery trees of the Early Harvest variety in the untreated rows was found to be as follows:

Row No.	Total trees	Trees showing true crown gall (Fig. 1)	Per cent
1	32	8	25.0
3	28	0	0
5	29	7	24.1
7	23	5	21.7
9	28	0	0
11	34	6	17.6
13	43	5	11.6
15	31	4	13.0
Total	248	Total 35	
Average per row	31	Average per row 4¾	Average 14.1

In the seven rows treated with sulphur the total number of the trees was 229, or an average of 32 trees per row. Here true crown gall was found on only one tree in row No. 2. Five trees, two in row No. 4 and three in row No. 14, were found with small, hard swellings at the union, as shown in Figure 2.

Of the Red Delicious variety there were 124 trees, two of which in the third row and one in the thirteenth row showed true crown gall. No crown gall was found in this variety in the rows treated with sulphur.

Of the Rome Beauty variety there were 41 trees, with true crown gall on one tree in the seventh, untreated, row.

Of the "Horse" variety there were 33 trees, of which true crown gall was found on one tree in the fourteenth row, treated with sulphur.

There were 285 of the two-year-old trees—those from which the scion wood was taken for the grafts. Examination of these trees showed that most of them, both in the sulphur-treated and untreated rows, showed more or less noticeable enlargement of the galls, 46 per cent and 64 per cent, respectively; one of the largest galls is shown in Fig. 3. On some of the trees, 24.5 per cent and 19 per cent, respectively, the galls showed very slight enlargement; on others the galls evidently have not increased; and in a few cases, 8 per cent and 7.3 per cent, respectively, the galls apparently disappeared. There was no definite indication of any effect, either of the original crown gall or of the treatment, on the stand or on the vigor and growth of these trees. Examination of the data obtained from the two-year-old trees shows that the treatment with sulphur may possibly have a somewhat retarding effect on the development of crown gall, although this is not certain. In this experiment fairly good sized holes were dug into which the trees were set. Owing to the digging in most cases very little or no sulphur was in a direct contact with the crowns of the trees and to this fact may be ascribed the slight, if any, effect of sulphur upon the crown gall.

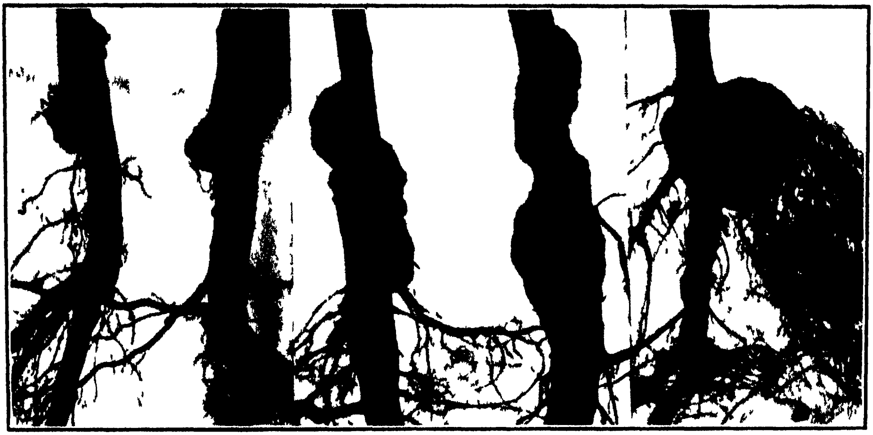

FIG. 1 FIG. 2 FIG. 3

FIG. 1. A small and hard swelling that in a few cases was found to develop in sulphur-treated rows on one year-old trees of Early Harvest variety. No soil was adhering to the surface of these swellings. One-half natural size.

FIG. 2. A typical crown gall found in the untreated rows on one-year old apple tree of Early Harvest variety. Some soil adheres to the surface of the gall when the tree is lifted even out of dry soil. One-half natural size.

FIG. 3. One of the largest crown galls found on two year old apple trees of Early Harvest variety. One third natural size.

From the data obtained with the one-year-old trees, it is evident that except in the case of Early Harvest there was not enough crown gall development on the trees grown in the untreated rows to admit of a conclusion in regard to the effect of the treatment upon the disease.

In the case of Early Harvest, however, the evidence is strong that the treatment is very effective in the control of the disease. Although the number of trees affected with true crown gall in the checks is not large, it is sufficient to be of definite significance when one considers the practically total absence of the crown gall in the treated rows.

Final proof, as well as many other factors of practical importance in connection with crown gall control by means of soil treatment with sulphur, such as the effective minimum application for various soils, methods of application, and the like, is, of course, to be obtained from further experiments.

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ROOT-ROT OF PEAS IN THE MIDDLE ATLANTIC STATES IN 1924

CHARLES DRECHSLER

During the spring of 1924, the writer participated in a survey of some of the pea-growing districts in Maryland, Delaware and New Jersey, the object of which was to ascertain the prevalence of root-rot and more particularly the relative importance of a number of parasites to which major damage had been ascribed. A season more favorable for such inquiry could scarcely have been chosen. An unusually wet April was followed by an excessively wet May, and that in turn by a June with a rainfall well above the normal. As May probably includes the period most important in the development of pea-root troubles in the region under consideration, the following passages in the meteorological reports from the Maryland and Delaware section, and from the New Jersey section, respectively, descriptive of the weather conditions prevailing during that month, may be of interest:

"May was markedly cool and unusually wet. . . . The average rainfall, 6.44 inches, was one and four-fifths times the normal and the greatest of record in May since 1889."[1]

"Subnormal warmth and excessive rains, which prevailed in April, continued throughout May, so that weather conditions, relatively, assumed an unprecedently unfavorable aspect. The low temperature, the amount and frequency of rain and consecutive cloudy days have rarely been equalled singly, and never in combination. It has probably been the worst spring since 1886, which was also cold and damp."[2]

Observations were begun May 15 in Talbot County, Maryland, where the more advanced fields had been blossoming for several days. Fields in Queen Anne County were visited the following day. On May 27 and May 28, inspections were made in a number of localities in Sussex County and Kent County, Delaware; and continued on May 29 in Cumberland County and Camden County, New Jersey. The survey was concluded in Carroll County, Maryland, on June 11, the crop here being well advanced, at a stage preceding readiness for harvest by about two weeks. With the exception of experimental plots near Cedarville, N. J., and at Arlington Farm, Va., as well as of a number of fields in Anne Arundel County, Maryland, devoted to the cultivation of fresh peas for the city market, all the tracts inspected were planted to peas apparently of the Alaska type.

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[1] Spencer, J. H. General Summary. Climatological Data. Maryland and Delaware Section. 29: 17. 1924.

[2] Noyes, G. H. General Summary. Climatological Data. New Jersey Section. 29: 17. 1924.

The principal forms of root disease were found represented in one or more fields in each locality visited. An infection of the type attributed by Jones[3] to the fungus described as *Fusarium martii* App. & Wr. var. *pisi* occurred rather widely, but at the same time quite sparingly. It was the only root infection found, for example, in a plot at Arlington Farm never before planted to peas, and was manifested here as a vascular trouble, the cortical tissue surrounding the woody core with its reddish brown discoloration, not being visibly decayed. By June 6, after the advent of warm conditions, the scattered individual plants attacked had stopped growing, their foliage was of a sickly yellow color, and incipient symptoms of wilting were becoming evident. Members of the genus Fusarium were richly represented also in isolations made from the greatly softened cortex of roots bearing an abundance of oospores of a fungus recently described[4] as *Aphanomyces euteiches* Dr., but their occurrence, under such circumstances, did not appear strikingly indicative of a parasitic relation.

In regard to species of Pythium, a very similar condition obtained. More than a hundred isolations referable to the genus were made, each from a different collection of diseased plants, except in such instances where the same collection yielded growths obviously belonging to separate species. From 6 to 8 distinct species are recognizable in the assortment, which will receive taxonomic treatment later in a more comprehensive account. At least 3 of these species represent types ordinarily encountered by the pathologist, and customarily referred to *Pythium debaryanum* Hesse, being distinguished by abundant aerial mycelium in culture, smooth oogonia, and subspherical sporangia or conidia. Over a score of isolations represented types with spiny intramatrical oogonia and poorly developed aerial mycelium, falling into 2 or 3 species referable to Artotrogus, a group usually regarded as a subgenus, but perhaps not undeserving of generic rank.

Although some isolations were made from discolored rootlets not showing evidence of being attacked by any other fungus, the most prolific source for cultures of species of Pythium was found in the cortical tissues of the stem and larger roots bearing the oogonia and oospores of *Aphanomyces euteiches*. In fields in which the latter fungus was common, the genus Pythium appeared to be found occurring more abundantly in secondary relationships than in directly parasitic ones. This condition is apparently not due to any lack of potential virulence, since nearly all of the smooth forms tried out so far, as well as one spiny form, which was derived from material collected at Hamburg, N. Y., attack cucumber fruits with great readiness—generally, a fair index of a moderate degree of pathogenicity. The remaining spiny species, on the other hand, fail to attack cucumbers, but, with one exception, are capable of developing in watermelon fruits when inoculated under the rind, such development requiring, in general, a relatively low degree of virulence. The widespread occurrence of even the most aggressively parasitic species of Pythium in dead organic matter has long been recognized, and it is evident that the tissues killed by *Aphanomyces euteiches* provides a more congenial substratum than the living parts.

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[3] Jones, F. R. Stem and root-rot of peas in the United States caused by species of Fusarium. Jour. Agr. Research 26: 459–476. Illus. 1923.

[4] Jones, F. R., and C. Drechsler. Root-rot of peas in the United States caused by *Aphanomyces euteiches* n. sp. In press; to appear in Jour. Agr. Research.

Mycelium of *Corticium vagum* B. & C. var. *Solani* Burt (*Rhizoctonia solani* Kühn) was frequently encountered in the softened cortex of diseased stem or main root. In most instances its occurrence, in conjunction with an abundance of oogonia of *Aphanomyces euteiches* gave grounds for regarding also its role more that of a secondary invader than of a primary parasite. Occasionally, to be sure, lesions of the type characteristic of its parasitism were found in the field. The fungus was not found to be regularly associated, however, with a very common but relatively innocuous condition, resulting from partial or complete decay of the cotyledons, which decay frequently extended a short distance over the adjacent portions of root and stem as a corrosion of the cortex.

Aphanomyces euteiches was found to be incomparably the most important primary cause of root-rot. In approximately one-fourth of the fields visited, infection by this Saprolegniaceous parasite was so thoroughgoing that not a single healthy plant could be located even when special search was made for individuals that might have escaped the disease. The underground parts of every plant were found involved in characteristic softening of the cortex, and, on some sandy soils, where the vines had become somewhat prostrate, the destruction of cortical tissue extended several centimeters up the aerial portions of the stem. Fields thus affected were readily recognized at a distance by the pale yellow color of the foliage. Microscopic examination revealed the oogonia and oospores characteristic of the parasite in the softened cortex of all underground parts large enough to be conveniently removed from the soil.

In approximately one-half of the fields inspected, the disease due to *Aphanomyces euteiches* was found in more moderate quantity, frequently being present in severe form only in situations unduly wet as a result of inadequate drainage or proximity to watercourses. To a considerable extent, its distribution appeared quite fortuitous, badly infected individual plants being intermingled in stands that were largely healthy. In the remaining fields, representing about one-fourth of the entire number visited, root-rot was either entirely absent, as far as could be determined, or present only in very small quantity. This condition was typical generally of land which had not been planted to peas before, or which was known not to have been in peas for many years. Here the diseased specimens could be located only by carefully searching for yellow vines, occurring singly here and there, or, perhaps, in widely scattered groups of two or three.

Some of the instances of extreme or very heavy infection could readily be related to a patent lack of proper rotation, as, for example, where fields had been planted to peas 3 or even 7 years in succession. As the necessity for rotation is quite generally understood by the majority of growers, such cases were not found especially frequent. In certain localities, where a 5-year rotation is being practised, 2 successive crops of peas are scheduled to follow 3 successive crops other than peas. A number of fields thus cropped to peas were among those showing extreme or very heavy infection. In one instance extreme infection was found in a field, which, according to information supplied by the owner, had not been planted to peas in recent years, but had been fertilized with vines from other fields. In general, the evidence appeared to indicate that while in most types of soil 3 years constituted a sufficiently long interval to permit the growing of a single crop of peas without much damage from root-rot, it was not altogether adequate where a second crop of peas was to follow the first, at least in seasons as favorable for the disease as that of 1924. A further reduction of the residual contamination of the soil with *Aphanomyces euteiches* by extending the period devoted to crops other than peas to 4 or 5 years, might prove advisable.

The influence of type of soil on the various manifestations of root-rot is not readily determined by observations of a single season. The destruction of cortex appeared to extend further up the stem above the ground line on loose open soil than on heavier compact soil. The centrifugal distribution from the original foci of infection would seem to progress more slowly in dense soil. Thus in the plot at the experimental substation at Ridgely, Maryland, the soil of which is of a less open texture than the soils usually devoted to pea-growing, the areas exhibiting root-rot in 1924 were found to correspond closely to similar areas observed in the previous season, the increase in size not being marked. It may be mentioned in this connection that most of the fields showing extreme infection were on the more porous types of soil. Although it is not difficult to understand why the open types of soil should be better adapted to the spread of the zoospores of the parasite, the distribution of the fungus on a larger scale could scarcely be effected directly by locomotion of the zoospore alone. On the other hand, seepage through the superficial layers of soil, or slow surface drainage of water abounding with swarm spores, such as might readily have occurred, for example, during the wet spell from May 8 to May 12, might provide means much more effective in distributing the fungus in porous soil than in compact soil.

Although the disease due to *Aphanomyces euteiches* occurred with more than usual severity, losses from this source were not as heavy as might have been expected. As is well known, diseased plants continue to develop in spite of the virtually decorticated condition of the underground parts as long as an ample supply of moisture is available in the soil, the reduced efficiency of the root system being expressed chiefly in the yellowish, sickly appearance of the vines. The worst effects do not show until dry conditions intervene, when the diseased root systems fail to maintain transpiration with the result that the plants wither and die. Owing to ample, well-distributed precipitation during the month of June, such wilting was prevented this season—an instance of the most destructive phase of a disease being obviated by a continuation of the very conditions that had encouraged its inception.

While, to be sure, the crop on the whole was not an unsatisfactory one, owing largely to fortunate weather conditions obtaining during the latter part of May and throughout June, the writer believes that the prevalence of the Aphanomyces disease brought about a material decrease in yield. Where the trouble appeared early and in extreme degree, the obvious reduction in vegetative vigor could scarcely have made for a maximum production of pods. In one known instance, a field so completely infected by May 15 that no healthy individual plant could be found, showed up so lacking in promise a few weeks later, that the vines were plowed under. Although this case may not be typical, it illustrates a kind of economic loss associated with the disease, quite apart from the more drastic loss brought on by intervention of droughty conditions.

Evidence that root-rot was present in the pea-growing districts north of Maryland and New Jersey, was provided by specimens of diseased peas sent to the Plant Disease Survey, which the writer had occasion to examine. Oogonia and oospores characteristic of *Aphanomyces euteiches* were demonstrated in variable quantity in almost all the material, thus establishing the presence of the parasite at New Haven, Connecticut; at Eden, Hamburg and Mt. Morris, in the eastern part of New York; at Mineola, on Long Island; and at Aspers, Drifton and State College, in Pennsylvania.

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A MARASMIUS PARASITIC ON SMALL GRAINS IN ILLINOIS

P. A. YOUNG

WITH FIVE FIGURES IN THE TEXT

While collecting data and specimens for the Illinois State Plant Disease Survey during the summers of 1923 and 1924, the writer found small mushrooms attached to the stems of wheat, rye, barley, quack grass, and an undetermined grass. At Abingdon, in central Illinois, an area of a few square rods in one wheat field showed mushrooms attached to half of the stalks. In all cases observed, the sporophores were attached within two inches of the surface of the ground as shown in Figure 3. The other collections consisted of only a few mushrooms each. The culms of the quack grass were green when the mushrooms were collected on them. The small grains were ripening and the mushrooms on them had dried up when the specimens were collected. No evidence of injury to crops was seen.

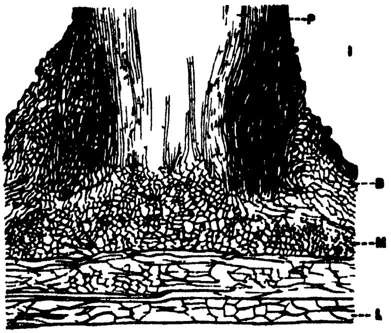


FIG. 1

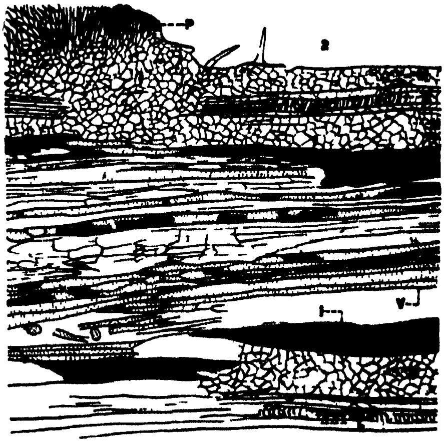


FIG. 2

FIG. 1. Section through base of stipe showing attachment to leaf sheath. P, thick-walled prosenchymatic cells of stipe, thin-walled cells inside. B, brown, unstained cells in base of stipe. M, mycelium and disintegrating host cells; black spots represent thick fragments of purple stained hyphae. L, inner side of leaf sheath. Slightly diagrammatic. × 100.

FIG. 2. Section through wheat stem showing mycelium around base of stipe and near the culm cavity. P, thick-walled prosenchymatic cells of base of stipe. M, mycelium and disintegrating host cells. S, spiral trachea. V, pitted tracheae. I, indefinite and broken cells, mostly pitted tracheae. L, inner layer of cells next to culm cavity. Slightly diagrammatic. × 40.

Though Cobb (1), Fulton (2), and Johnston(3 and 4) report species of Marasmius and some other agarics on sugar cane, the only published records of agarics occurring on small grains in the central part of the United States that the writer has seen are two brief notes by Tehon (5, 6) in which he reports the presence of the fungus considered here. Mushrooms parasitic on chlorophyll-containing tissues are comparatively rare.

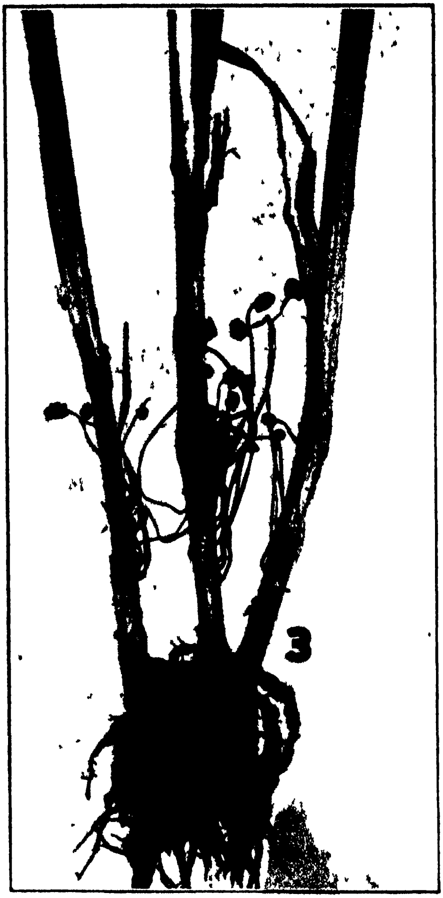
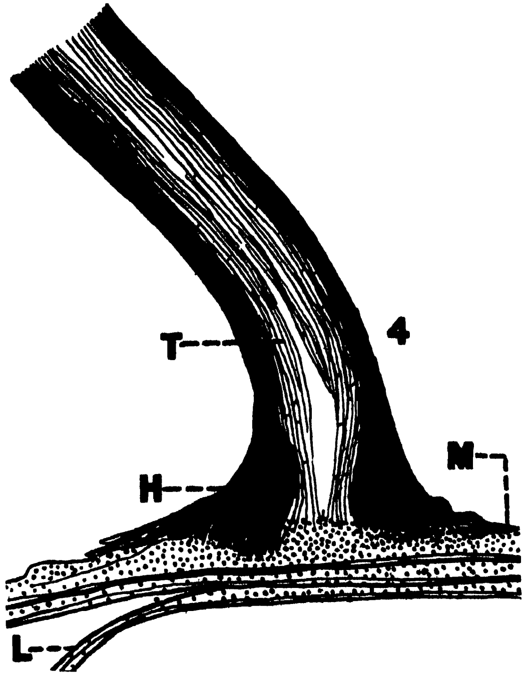


FIG. 3. Photograph of Marasmius sporophores attached to bases of wheat stems. × about 1½.

A characteristic dry piece of wheat culm bearing several mushrooms was soaked in lacto-phenol fixer for 3 days, imbedded, sectioned, and stained with Pianeze III B. The sections showed the mycelium deep in the host tissues (Fig. 2). A section of a young sporophore which had not yet broken through the epidermis is represented in Figure 5.

The five figures show the origin and attachment of the sporophores.



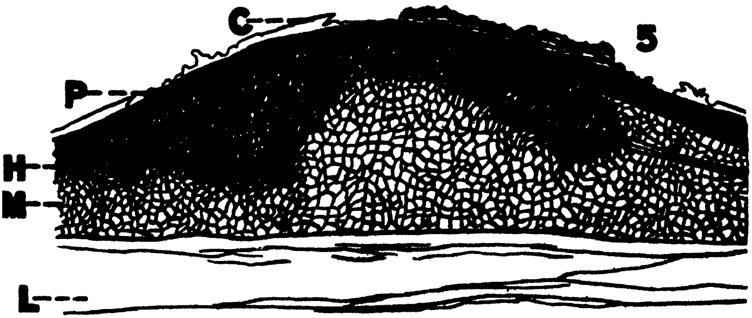


FIG. 4. Lower part of stipe showing swollen base and attachment to leaf sheath. T, thin-walled prosenchymatic cells. H, thick-walled prosenchymatic cells. M, mycelium. L, lower side of leaf sheath. Diagrammatic. × about 55.

FIG. 5. Early stage in the development of a young sporophore; not yet broken through the primary cortex, P. C, cuticle. H, thick-walled prosenchymatic cells. M, mycelium. L, lower cells of sheath. Diagrammatic. × about 135.

Note. All drawings from longitudinal sections through wheat stem. All outlines made with projection machine.

Only traces of the host tissues stain green and remain distinct below the sporophores. They are usually entirely decomposed and their space occupied by fungous tissues. Judging from the appearance of the sections, the mycelium must have developed while the host was still green.

Since this fungus does not agree with the descriptions of any of the species of Marasmius given in Volume 9 of the North American Flora, it is described as a new species. Only a small number of spores was seen. *Marasmius androsaceus* (L.) Fr. in Roumeguère's Fungi selecti exsiccati No. 6943 (species No. 153 in Vol. 9 of the N. A. F.) resembles the new species most. It is a saprophyte on fallen leaves in woods. The new species is described as follows:

Marasmius tritici, n.sp. Figs. 1–5.

Pileus membranous, convex to campanulate, depressed at center, 2–7 (usually 3–4) mm. broad; surface ocraceous to ferruginous, slightly pulverulent, margin distinctly sulcate; lamellae adnate to collar, slightly decurrent on collar, equal, whitish; spores 6–7 × 3–4 microns; hymenium layer 10–25 microns wide, basidia 3–4 microns wide; stipe brittle and sulcate when dry, dark brown, glabrous, shining, 2–4 cm. long and 0.3–0.5 mm. in diameter, hollow. Parasitic on culms of *Triticum vulgare* L. (smooth, red fall wheat), collected at Abingdon, Ill., July 12, 1924. Type specimen is No. 18116 in the herbarium of the Illinois State Plant Disease Survey and in the herbarium of the University of Illinois. Other collections are: No. 17332 on Red Wave wheat, Worden, Ill., 6–19–23; No. 17832 on *Secale cereale* L., Savanna, Ill., 7–16–24; No. 18060 on *Hordeum vulgare* L., (six-row barley), Wheaton, Ill., 7–31–24; No. 18198 on *Agropyron repens* Beauv, Wheaton, Ill., 7–31–24; and No. 5779 on an undetermined grass, Ridgway, Ill., 6–12–24.

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WATERMELON INTERNAL BROWNING

W. W. GILBERT AND ERNST ARTSCHWAGER

WITH FIVE FIGURES IN THE TEXT

An interesting case of internal browning in watermelons, apparently comparable with the internal brown-spot of the potato and the Baldwin spot of the apple, was recently brought to our attention by Mr. Wm. E. Lewis, Assistant Marketing Specialist of the Bureau of Agricultural Economics. The melons in which the trouble was found were grown at Burroughs, Ga., and constituted about 5 per cent of the crop on an area of about 225 acres.

Instead of the areas of dead, brown, pithy cells being scattered through the entire fruit as is the case with the apple and the potato troubles, they are confined to a peripheral ring one-fourth to one-half inch or more in width in the rind beginning about one-eighth to one-fourth inch inside the skin of the melon, as shown in the accompanying photographs of cross-sections of parts of affected melons (Fig. 1, a and b).

The first indication of this condition is exhibited by small rounded or irregular diseased areas which, at first only watersoaked in appearance, soon become pithy and discolored. In mild form, the spots are separate and have a diameter of one-eighth to one-half inch; in severe cases they form an almost continuous layer of brown tissue one-fourth to one-half inch or more in thickness and extending around the entire melon. Where the injury is most pronounced, there appears on the outside of the melon a coarse mottling or dotting of rather bright yellow, rounded spots one-half inch or more in diameter scattered over the normal green of the Tom Watson melon.

The melon rind, which is the seat of the injury, consists of an epidermis, a cortex, a sclerenchymatous cylinder and the peripheral tissue of the fruit flesh (Fig. 1, c). The epidermis is single-layered and topped by a heavy cuticle which extends inwardly between the anticlinal walls of the epidermal cells. Next to the epidermis is a narrow cortex about .2 mm. in width. The cells comprising this layer are small, thin-walled and somewhat flattened tangentially. The innermost layer of these cells abuts abruptly on the sclerenchyma sheath. The latter is composed of four or five layers of small irregular cells which are strongly lignified and perforated by numerous simple pits. The transition from sheath to the peripheral tissue of the fruit flesh is less abrupt than that between cortex and sheath. The cells grow perceptibly larger and elongated in the radial plane. Embedded in the peripheral tissue of the fruit flesh are small collateral bundles, the extremities of the vascular bundle system of the melon.

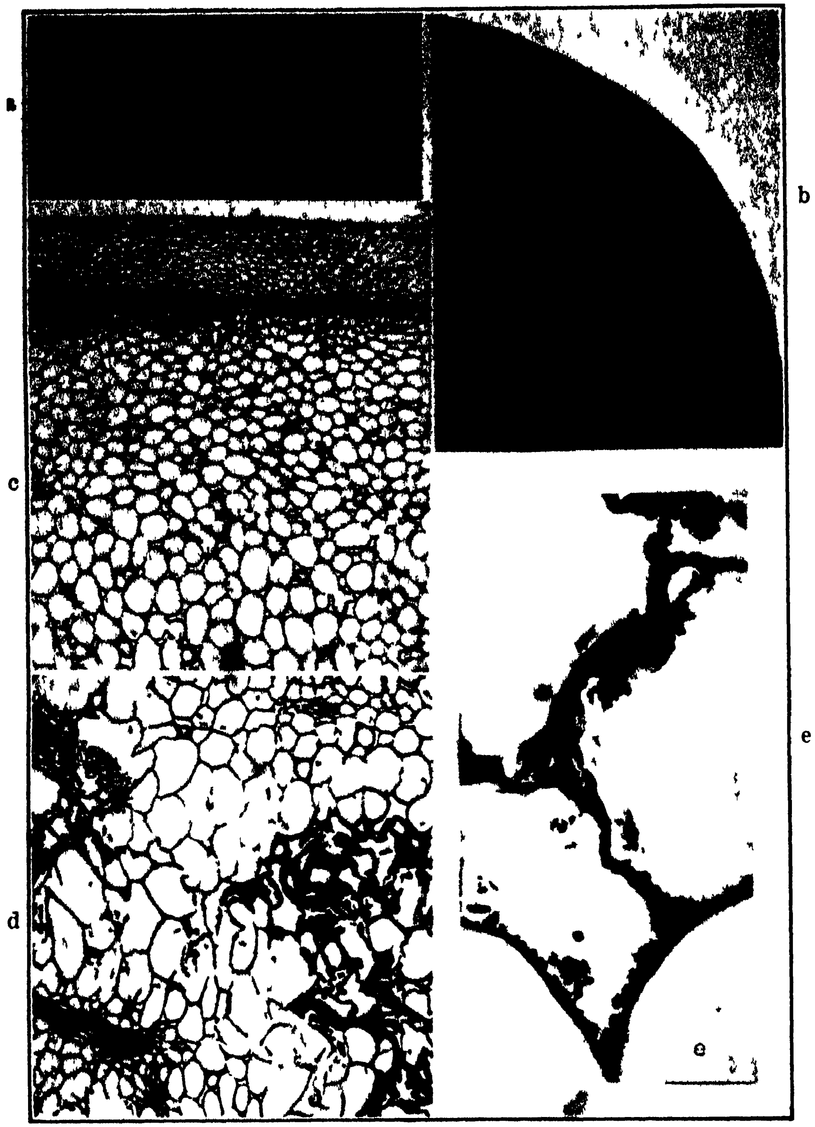

FIG 1 a Section through melon rind showing band of necrotic tissue —b Section through melon showing relation of diseased tissue to the melon as a whole —c Cross section through melon rind showing epidermis, cortex, sclerenchymatous cylinder and peripheral fruit flesh × 56 —d Cross section through peripheral fruit flesh showing necrotic areas Notice that the vascular bundles are normal × 56 —e Enlarged view of necrotic cells × 400

The diseased areas described above develop in the peripheral fruit flesh or inner part of the rind. The injury is confined to the parenchyma (Fig. 1, d); the vascular bundles always appear normal, unless by chance diseased parenchyma cells border directly on a vascular bundle. In the early stages of the disease, exhibited by the water-soaked condition of the tissues, the walls of the cells are already partly or entirely lignified. Lignification is evident at first in the middle lamella and only later the entire wall and content become affected. The walls appear swollen and lamellate especially in the region of the intercellular spaces. The latter become filled with a granular content which is later also seen in the lumen of the cells (Fig. 1, e).

In advanced stages, when discoloration becomes visible to the naked eye, the cells of the diseased areas have collapsed. Both walls and content have a brownish color characteristic of necrotic cells. In the specimen tested there was no apparent deleterious effect on the flavor of the melon, although it is quite probable that the marketability of such melons is impaired.

The cause of this abnormal condition has not been determined, but a period of four or five weeks of very dry weather which occurred while the melons were maturing is thought to have had some relation to the trouble.

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RHIZOPUS ROT OF PEACHES

H. W. ANDERSON

Brown rot of peaches has long been regarded by pathologists and orchardists as the only important rot in the orchard and in transit. Those handling the fruit in market are usually not sufficiently acquainted with diseases to distinguish the various types of rot. Consequently rejections of the fruit by commission men is usually attributed to brown rot. The inspectors of the Bureau of Agricultural Economics, however, distinguish between brown rot and Rhizopus rot and it is interesting to note that Rhizopus rot is very frequently the only rot reported by the inspectors.

Recently, I looked over the reports on peaches of the Bureau of Agricultural Economics for the state of Illinois, *i. e.*, of shipments originating in this state. The markets where the inspections were made were widely separated and included such cities as Milwaukee, Minneapolis, St. Louis, Detroit, Columbus, O., Memphis, Cleveland, Cincinnati, Pittsburgh and Kansas City. This wide distribution eliminated the error of one inspector being more familiar with this particular disease and thus favoring it during inspection. The inspections include three years, 1922, 1923 and 1924. The result of the tabulation was very surprising to me.

TABLE 1.—*The frequency of peach rots in shipments of peaches from Illinois*

Year	Total no. cars	No decay	Rhizopus rot	Brown rot
1922	50	19	20	21
1923	29	8	15	13
1924	25	9	9	7
Total	104	36	44	41

This table shows that Rhizopus rot was reported more frequently than brown rot and, since it is more destructive than brown rot, the loss from Rhizopus rot in transit, storage and market is probably much greater.

Inspection of peaches on local markets during the last three or four years has convinced the writer that Rhizopus rot is more frequently present and more to be feared than brown rot. The fungus (*Rhizopus nigri-*

cans) travels with great rapidity through the baskets, especially in the bottom, and attacks both injured and uninjured fruit. The mycelium penetrates the flesh very rapidly, resulting in complete decay within a day or two. For example, four bushels of sound Hale peaches were shipped from southern Illinois and left in a cool room for two days. They were in transit (express) one and a half days. At the end of this period (three and a half days) 30 per cent of the peaches were rotted. All the diseased peaches were removed and the healthy ones were placed in a clean basket. At the end of two days the peaches were again examined and fully half were more or less rotted. The fungus had produced enormous quantities of mycelium which had grown not only over the peaches but over the inner surface of the basket and in the spaces between the fruit. Wherever it came in contact with the surface of the peach the skin showed numerous small watery spots where decay had started.

Ordinarily this rot is not a serious factor in the orchard, but during the past season, an unusually wet one, several reports were received of serious damage to fruit on the trees. The growers were alarmed because they were not familiar with the fungus. In these orchards perfect control of brown rot had been secured by the use of dry mix lime and sulfur, but this treatment had not controlled the Rhizopus rot. The prevalence of this rot in the orchard may in part be explained by the fact that cracked fruit, due to weather conditions, was unusually abundant in most orchards this season.

The relation of temperature to the development of this rot is interesting and may indicate a possible method of control. Harter and Weimer[1] have studied the effect of various temperatures upon the germination of the spores and the growth of mycelium of *Rhizopus nigricans*. They show that growth is very limited below 7° C. (47° F.), but that at 15° C. (57° F.) growth is very rapid. It is evident that little loss would be experienced in transit provided the temperatures could be kept low enough, but this is quite difficult. Inspectors report more decay at the top of the car load and near the door than in other parts of the car. Since the temperatures at these points are usually four or five degrees above those of other parts, it is evident that a rise of a few degrees makes considerable difference in the amount of Rhizopus rot. The temperature at the top of the load averages nearly 47° F. according to the reports of the inspectors. This is probably near the minimum temperature of the car in transit since the fruit is loaded warm and the temperature of the car decreases gradually until it reaches its destination. After the fruit leaves the car, unless it is immediately placed in cold storage, the temperature increases rapidly and soon approaches the optimum for the development of *Rhizopus nigricans*. It is evident, therefore, that while the low temperature of the refrigeration car materially retards growth, at no time is the temperature below the minimum for the germination of the spores and the growth of the fungus. A more rapid cooling of the fruit and care in icing the cars may aid materially in reducing the amount of damage.

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[1] Harter, L. L., and Weimer, J. L. Some physiological variations in strains of *Rhizopus nigricans*. Jour. Agr. Research **26**: 363–371. 1923.

Weimer, J. L., and Harter, L. L. Temperature relations of eleven species of Rhizopus. Jour. Agr. Research **24**: 1–40. 1923.

It is the opinion of the writer that more attention should be given by pathologists to this rot and a study should be made of methods of control in transit and storage. Brown rot in properly sprayed or dusted orchards is no longer a serious factor under Illinois conditions, but great losses are being experienced on account of Rhizopus rot in transit and market.

UNIVERSITY OF ILLINOIS,
URBANA, ILL.

ABSTRACTS OF PAPERS PRESENTED AT THE EIGHTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, PENTICTON, BRITISH COLUMBIA, AUGUST 26 TO 29, 1924

SYMPOSIUM

Virus diseases and their relation to potato seed certification. Discussion led by B. L. RICHARD, Utah; B. F. DANA, Washington; J. W. EASTHAM, British Columbia, and C. W. HUNGERFORD, Idaho.

Physiology of zoospore formation among certain species of Phytophthora. F. B. COTNER.

Witches broom of potatoes. B. F. DANA and C. W. HUNGERFORD.

Sunflower wilt. H. E. MORRIS.

Evaluation of loss from killing diseases in young forests. E. P. MEINECKE.

Detection of spoilage in berry products. CARL R. FELLERS.

Water core in apples. D. F. FISHER.

Black rot and Myxosporium canker of apple and pear in the Pacific Northwest. S. M. ZELLER.

Investigative work on white pine blister rust in the Pacific Northwest for 1923. J. S. BOYCE.

During 1923, circumstantial evidence was such that for practical purposes it must be concluded that blister rust can spread directly from western white pine to Ribes, a distance of 110 miles, and probably much farther. Numerous infections on *Ribes nigrum* were found in the interior of Washington and British Columbia many miles from the nearest 5-needle pines, and infection was present on *Ribes bracteosum* at Namu, and *Ribes nigrum* and *Grossularia divaricata* at Bella Bella, on the coast of British Columbia, 80 and 110 miles, respectively, north of the known range of 5-needle pines. So far it has been impossible to obtain any conclusive evidence of the disease overwintering on Ribes.

The development of the rust on Ribes served to emphasize the extensive importance of *R. nigrum* in the distribution and spread of the disease. This species is by far the most highly susceptible occurring in the Northwest. At long distances from pine infections and where climatic conditions are less favorable to the development of blister rust, as in the interior of Washington and British Columbia, initial infection was practically confined to *R. nigrum.*

Some facts about Loganberry "dwarf." S. M. ZELLER.

This disease, which has many of the symptoms of bramble streak as found in blackberries in the East, is characterized by short internodes of the canes, several small buds at each node, and small, slightly yellowed, obovate leaflets. Streaking of the canes has never been observed. Transmission of the disease has not been demonstrated, but evidence shows that the disease is not propagated by layering, for affected tips do not root.

A case of Verticillium wilt (Blue-Stem) of black raspberry in Oregon. S. M. ZELLER.

One acre of 3-year-old Plum Farmer black raspberries on land planted to potatoes the year before had about 52 per cent of the plants dead or dying during the late fall of 1923–24. Mungers on the same soil which had not been in potatoes were doing well after five years. Cultures of a strain of *Verticillium alboatrum*, Reinke and Berth, were obtained from 49 out of 51 of the affected Plum Farmer plants.

A preliminary report of the uredinales of Washington. J. W. HOTSON.

This article, which will appear shortly in the Biological Publications of the University of Washington, is an annotated list of the rusts of the state of Washington. It is based on over 2,000 collections made in various parts of the state. These are distributed among nearly 200 species.

Since many of the collectors of Washington rusts do not reside in the state, it is highly probable that many collections have been omitted from this preliminary list. For this reason a supplementary list will be published as soon as the number of new collections will warrant it. If this report should come to the notice of any person who has made collections in any part of the state of Washington, the author will be glad to receive samples of such collections.

Serious blossom blight in Pacific Northwest orchards due to a species of Monilia. H. P. BARSS.

Much economic loss is annually experienced in orchards of Western British Columbia, Western Washington, Western Oregon and perhaps farther south due to the blighting of blossoms and killing of spurs, accompanied sometimes by cankering and girdling of smaller branches and twigs and followed by a negligible amount of fruit rot caused by a species of Monilia similar to *Sclerotinia cinera* (Bon.) Wor. but, in the view of the author, distinct from the latter because of its different life-history, growth characters and spore morphology. The fungus was first studied some years ago by Mr. G. B. Posey under the direction of the author, in an investigation, the results of which appear in an unpublished thesis. The name *Monilia oregonensis* Barss and Posey has been assigned to it. Apricots, sour cherries, sweet cherries, prunes, peaches and pears are the principal hosts. Quince and apple fruit has been found infected. The fungus winters in the blighted parts, producing olivaceous spore-tufts in winter and spring. Apothecia are unknown and could not be produced by the conditions favorable for apothecium formation in *Sc. cinerea.*

Plant pathology in California. RALPH E. SMITH.

California is quite unique in its diversity of natural conditions and the variety of its crops. Most of its agriculture is carried on under rather artificial conditions. Numerous major plant disease problems occur, many of them still entirely unsolved. Probably all of the usual fungus diseases of the important crops of California have been introduced, but many of them are unknown or of minor importance on account of the rainless summers. Excellent control of such troubles of this sort as attain economic importance is quite uniformly obtained. The more serious phytopathological problems are to a large extent of two other classes.

1. Apparently, or so-called "physiological" disorders of a very specific nature. Typical examples, "blossom end rot" of tomato; "black end" of pear; "little leaf" of deciduous fruits and other trees; "tip burn" of lettuce; "exanthema" of many trees. Many or all of these show an apparent relation to soil moisture and nutritional conditions and no evidence of parasitism. Some of these diseases are discussed.

2. Troubles in which a specific parasite probably occurs, but where parasitism depends largely upon obscure environmental factors. Examples discussed.

Results of experiments in 1924 with various chemical dusts for smut control in wheat. H. P. BARSS.

Fall planted rod row tests with heavily smutted wheat (1 part spores of *Tilletia tritici* to 77 parts of grain by weight) using various samples of chemical dusts thoroughly applied at the rate of 2 ounces per bushel showed that the best control was obtained by brands of copper carbonate of standard fineness containing 50 per cent or more of copper, that next in efficiency were the types of copper carbonate containing 17 to 21.5 per cent of copper followed by Seed-o-San, Semesan and Corona 620, all organic mercury compounds. Nickel carbonate was much less effective. Furfural (1 to 400) used as a dip proved entirely ineffective. Two mercury compounds proved utterly valueless applied as a dust. Complete smut control was not obtained in the case of any material used on wheat smutted at the 1 to 77 rate, yet with wheat smutted at the rate of 1 to 500, high grade copper carbonate at 2 ounces per bushel gave complete smut control, while none of the other dusts gave less than 23 per cent smut heads under similar conditions. At 1 to 1,000, however, copper carbonate with 50 per cent copper, copper carbonate with 21 per cent copper and Semesan all gave complete or practically complete control.

Conclusions from four years' tests of various methods of seed treatment for bunt control in Idaho. CHAS. W. HUNGERFORD.

Extensive field tests carried on in coöperation with farmers in northern Idaho as well as numerous plot tests have shown that copper carbonate dust of standard fineness and containing at least 50 per cent copper, applied at the rate of two ounces to the bushel, has not given as good control as the standard bluestone and salt treatment. Three ounces to the bushel have given nearly as good results as bluestone and salt. Copper carbonate dusts, containing less than 50 per cent copper, were not so effective unless used in larger amounts. Semesan, four ounces to the bushel, was as effective as copper carbonate at three ounces. Three ounces of nickel carbonate was not so satisfactory.

Experiments for the control of bunt in spring wheat in 1924 showed that copper carbonate (six different brands used both two and three ounces to the bushel), Corona 640, Corona 640S, semesan dust and semesan and uspulun liquid treatments reduced the amount of smut to less than one per cent in each case while the checks developed over 12 per cent. Furfural of various concentrations gave practically no control and its use resulted in very serious injury to germination.

The application of certain organic mercury compounds in plant pathology. GEO. H. GODFREY.

The organic mercury seed disinfectants were initiated in Europe about twelve years ago with the development of uspulun, a compound of the coal tar product phenol, with a salt of mercury. Its superiority over the inorganic salts as a disinfectant in the field of agriculture became immediately evident, and a great deal was written regarding it in the European literature. Its introduction to America was delayed only by the war. Recently it has been introduced generally in America, through the Experiment Stations. Simultaneously other organic metallic salts have been developed and introduced in an experimental way.

Results secured in Europe and America show uspulun, the one longest established in the literature, to have a rather wide range of usefulness in plant pathology. It is an efficient disinfectant, with good penetration, so that it kills more than the merely superficial organisms. It does not, within a reasonable margin of the recommended strength or time of application, injure the seed. It protects seed from decay by soil organisms, this protection extending even through the seedling stage, so that a better stand is secured. This is particularly the case where soft seed or seed of low germination is under consideration. Damping off of seedlings is often prevented by a seed treatment or a soil treatment about the young seedlings, or a combination of both. Some diseases such as brown-patch of lawns and golf greens have been efficiently controlled by application of uspulun. Results reported by various experimenters in America show without doubt that the organic salts of mercury are deserving of thorough consideration on the part of the plant pathologists in America.

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STUDIES ON THE BEHAVIOR OF FUSARIUM CROMYOPHTHORON IN CARBOHYDRATES, GLUCOSIDES, PROTEINS AND VARIOUS DECOCTIONS, WITH A DISCUSSION ON THE "ISOMETABOLIC POINT" OF SUBSTANCES

CHRISTOS P. SIDERIS[1]

INTRODUCTION

There is some confusion among students of microbiological physiology on the nature of the metabolic products released in the solution as a result of the reaction of various fungi and bacteria with different culture media. This confusion appears quite often in the literature in the statements of different investigators on the behavior of the same organism.

The writer, by careful technique, has been able to study the nature of certain of these metabolic products and particularly that concerned with the changes produced in the hydrogen-ion concentration of different culture media during the growth of *Fusarium cromyophthoron* Sid. (16). He found, by frequent determinations of the changes of the hydrogen-ion concentrations, that the behavior of the organism was constant under any set of conditions, and was able also to reconcile the contradicting views of the different investigators. He found, in other words, that Fusaria may change the reaction of their substratum either by increasing or decreasing the hydrogen-ion concentration, the nature of the reaction depending (a) on the initial pH value of the culture solution, (b) on the chemical nature of the nutrient substance employed and (c) on the age of the culture.

This paper is concerned (a) with an analysis of the behavior of *F. cromyophthoron* in different culture media, (b) with a reconciliation of the views of previous investigators on the behavior of a number of organisms in different culture media, and (c) with a discussion on the "isometabolic point" of substances, *e.g.* of the initial pH value of the culture solution which may be changed slightly or not at all by the reaction of the metabolic products of an organism during the assimilation of a certain nutrient substance.

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[1] The writer is indebted to Professors A. R. Davis and D. R. Hoagland for reading the manuscript and for many helpful suggestions.

REVIEW OF LITERATURE

There are a number of excellent publications concerned with the behavior of various fungi and bacteria in different culture media. Those of quite recent origin are particularly concerned with the changes produced by microorganisms in the H-ion concentration of their culture media; it is these which will be considered in this presentation.

In a review on the behavior of different Fusarium species and other organisms in different culture media, we find that organisms in general behave differently at different stages of their development. For instance, Young and Bennett (22), in growing *F. oxysporum* Scht. in culture media at different initial pH values, found that

> . . . the reaction of the culture solution in which *F. oxysporum* was grown continued to become acid until an H-ion concentration of pH 3.6 was reached, then turned toward alkalinity and growth continued until all the organic compounds were broken up and an H-ion concentration of pH 8.4 was reached.

Johnson (7), in studying the resistance of seven molds, including *F. bullatum* and *F. oxysporum,* found that

> . . . the optimum reaction for the molds lies more to the acid side of the neutral point than toward the alkaline, practically all of them show nearly a maximum growth as low as pH 2.0, while they are cut off quite sharply at pH 8.7. The reaction of all the solutions was changed toward a more alkaline value except the neutral solution with *F. bullatum.* In no case did the molds reach the pH values which were found to inhibit their growth in the studies on the effect of hydroxyl ions.

Sideris (18), in his studies on the behavior of *Fusarium cromyophthoron* in onion decoctions at different initial H-ion concentrations, found that the organism changed the reaction of all the cultures except that at pH 5.5; those between pH 3.0 and 5.5 were changed toward the latter value by a decrease of their H-ion concentration, and those between pH 7.5 and 5.5 were also changed toward the latter value by an increase of their H-ion concentration. Weimer and Harter (10) report that the following organisms changed the H-ion concentration of a 10 per cent glucose solution as follows: *Fusarium acuminatum,* pH 3.97 to 3.89; *Diplodia tubericola,* pH 3.97 to 3.66; *Rhizopus tritici,* pH 4.94 to 2.11; *Mucor racemosus,* pH 5.44 to 3.09; *Sclerotium bataticola,* pH 4.54 to 4.21; *Penicillium sp.,* pH 5.70 to 4.37; *Botrytis cinerea,* pH 4.99 to 3.24; and *Sphaeronema fimbriatum,* pH 5.19 to 5.50. Harter and Weimer (4) report that *Rhizopus tritici* Saito changed the initial pH value of culture solutions of different nutrient substances as follows: string bean decoction, pH 4.80 to 7.99; prune decoction, pH 3.91 to 3.53; Irish potato decoction, pH 5.60 to 7.80; carrot decoction, pH 4.98 to 4.47; turnip decoction, pH 4.83 to 4.26; sweet potato decoction, pH 5.05 to 3.31; Czapek's solution, pH 4.14 to 2.47; Pfeffer's solution, pH

3.48 to 2.61; Richard's solution, pH 3.36 to 2.46, and beef bouillon pH 8.04 to 8.52. MacInnes (9), in a study on the behavior of *Fusarium sp.*, found that

> . . . as growth proceeds the H-ion concentration of the solution is changed by the organism, those on the acid side tending to become more alkaline and those on the alkaline side more acid.

Paine and Chaudhuri (12) report that *Bacillus solanisaprus* Harr. changed the initial pH of an Irish potato decoction from pH 6.4 to 4.8, and *Bacillus atrosepticus* v. Hall, the same decoction from pH 6.4 to 7.2. Rosen (15) reports that his organism, *Bacillus alboprecipitans* Rosen, changed the pH value of beef broth from 7.2 to 8.0. Wolf (21) states that some pathogenic bacteria grown in media containing pectin changed the pH of the solution as follows: *Bacillus carotovorus* Jones, pH 7.4 to 5.2; *Bacterium tabacum,* pH 7.4 to 6.4; *Bacterium angulatum,* pH 7.4 to 6.4; *Bacterium sojae,* pH 7.4 to 6.8, and *Bacterium campestre,* pH 7.4 to 6.0. Dernby (3), in studying the behavior of a number of bacteria at different hydrogen-ion concentrations, expresses the opinion that bacteria may be classified into distinct groups according to the changes they produce in the H-ion concentration of their substrata.

The above citations on the behavior of a number of organisms, and particularly of Fusaria, lead to the conclusions (a) that different organisms may or may not behave alike in the same or different nutrient substances, (b) that the tendency in the majority of the organisms is to increase the H-ion concentration of the solution in cultures containing available carbohydrates and decrease it in those containing available proteins, and (c) that the reactions produced in certain vegetable and meat decoctions depend to a considerable extent on the initial hydrogen-ion concentration and the ratio of available carbohydrates to proteins contained in the particular decoction.

METHODS OF EXPERIMENTATION

The methods employed in these studies aimed to yield a certain understanding of the operation of the various factors which initiate the changes in the H-ion concentration of certain culture media during the growth of some microorganisms. For the purpose, substances of a chemically different constitution were used for the growth of the organism with the expectation that the resulting metabolic products would react differently on the hydrogen-ion concentration of the culture solution. The different nutrient substances employed belong chemically to the following classes of substances: carbohydrates, glucosides, proteins, and animal and vegetable decoctions. They were used in different amounts for the preparation of the different culture media For instance, for the preparation of dextrose solutions, 2 per cent dextrose was used; for starch solution, 1 per cent potato starch; for pectin solution, 0.6 per cent pectin; for amygdalin solutions, 1 per cent amygdalin; for peptone solutions, 1 per cent peptone; for beef extract solutions, 1 per cent beef extract; and for onion decoctions, 20 per cent onions (18). The different nutrient substances were prepared into culture media by placing the amount designated in a solution of inorganic salts of the following proportion: distilled water 1000 cc., $MgSO_4$ 2.12 grams, $Ca(NO_3)_2$ 0.71 grams, KH_2PO_4 1.36 grams, and $Fe(NO_3)_3$ 1 cc. of a 5 per cent solution. The different cultures were adjusted to definite H-ion concentrations with additions of 0.2 normal HCl and KOH.

The apparatus devised by the writer (17) and used in other studies was employed, also, for the growth of *F. cromyophthoron*. The determinations of the changes in the H-ion concentration were made by the colorimetric method of Clark and Lub (2). The volume of culture solution removed from the flask for the determination of the H-ion concentration was in every case between five and ten cubic centimeters. With heavily colored solutions, the H-ion concentration was estimated by an Hildebrand hydrogen electrode (5).

BEHAVIOR IN CARBOHYDRATES

The carbohydrate substances, used for the growth of *F. cromyophthoron* and the study of the resulting changes in the hydrogen-ion concentration of the culture solution, were dextrose, potato starch and pectin.

Dextrose, a hexose sugar, is well known to all students of microbiology for its availability to a greater number of fungi and bacteria than any other known sugar. Certain of the metabolic products released in the culture solution by different micro-organisms during the assimilation of dextrose are alcohols and various organic acids. Certain organisms are quite specific in the production of these substances.

The assimilation of dextrose by micro-organisms is not thoroughly understood, due possibly to insufficient experimentation. It is not known whether the entire dextrose molecule penetrates the cell membrane and enters the cell as it is, or is broken into simpler substances and taken as such before penetration. With animal cells, we have the information that both dextrose and levulose molecules diffuse through the cell membrane without undergoing, previously, any changes (10). The procedure which micro-organisms employ for the assimilation of dextrose constitutes quite an important question, because it is related with the resistance of the protoplasm in high concentrations of various organic acids and alcohols. Although experimenters have been able to produce, by means of enzymes, organic acids and alcohols similar to those obtained by the reactions of the living organism, nevertheless they have not been able to settle satisfactorily the question on the assimilation of the dextrose molecule.

The assimilation of potato starch or pectin by micro-organisms is more difficult to understand than that of dextrose. Either potato starch or pectin, before they become assimilable, must be broken into simpler compounds, *e.g.*, hydrolyzed to dextrose in the case of the potato starch, or to some pentose sugar in the case of pectin.

The reactions produced in the various carbohydrates by the organism are recorded in tables 1, 2, and 3.

TABLE 1.—*Changes produced in the initial pH of a 2 per cent dextrose solution by F. cromyophthoron, from October 15 to November 15, 1923*

Date 1923	Culture solutions of different initial pH					
	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0
10/15	3.0	4.0	5.0	6.0	7.0	8.0
10/25	3.6	3.8	4.4	4.8	6.0	6.3
10/28	3.8	3.8	4.0	4.0	5.3	5.8
10/30	3.8	3.8	3.8	4.0	5.0	5.5
11/5	3.8	4.5	3.8	4.8	4.8	5.4
11/9	4.2	4.8	4.0	5.6	4.6	5.4
11/15	5.0	5.4	4.8	6.0	4.8	5.6

TABLE 2.—*Changes produced in the initial pH of a 1 per cent potato starch solution by F. cromyophthoron, from October 15 to November 15, 1923*

Date 1923	Culture solutions of different initial pH					
	pH 3.5	pH 4.5	pH 5.0	pH 6.0	pH 7.0	pH 8.0
10/15	3.5	4.5	5.0	5.6	6.8	7.8
10/20	3.9	4.2	5.0	5.5	6.4	7.5
10/25	4.2	4.4	5.0	5.3	5.8	6.8
10/30	4.4	4.6	5.3	5.5	5.8	6.4
11/5	4.6	4.9	5.6	6.0	6.4	6.6
11/10	4.9	5.2	6.0	6.3	6.6	7.0
11/15	5.2	5.6	6.2	6.5	7.0	7.2

TABLE 3.—*Changes produced in the initial pH of a 0.6 per cent pectin solution by F. cromyophthoron, from October 15 to November 15, 1923*

	Culture solutions of a different initial pH					
Date 1923	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0
10/15	3.0	4.0	5.0	6.0	7.0	8.0
10/20	3.2	4.4	5.2	6.4	7.0	7.6
10/25	3.8	5.0	5.8	6.6	6.8	7.2
10/30	5.0	5.6	6.2	6.6	6.6	7.0
11/5	5.8	6.2	6.4	6.4	6.5	6.8
11/10	6.2	6.4	6.5	6.5	6.6	6.8
11/15	6.2	6.8	7.0	7.2	7.4	7.0

Explanations on the Behavior in Carbohydrates

The results in tables 1, 2, and 3 indicate that the products released during the assimilation of dextrose, potato starch, and pectin by *F. cromyophthoron* may increase or decrease the acidity of the culture solution, the reaction depending on the initial pH of the culture and the chemical nature of the nutrient substances employed.

The behavior of *F. cromyophthoron* in dextrose solutions at different initial pH, in table 1, is manifested by an increase of the hydrogen-ion concentration in the cultures between pH 8.0 and 4.0, and by a decrease in those between pH 3.0 and 4.0. It becomes obvious from the behavior of this organism that the "isometabolic point" of dextrose lies at or near pH 3.8. This pH value suffered only slight changes by the reaction of the metabolic products during the assimilation of dextrose by *F. cromyophthoron,* while other initial pH values, either above or below this point, were changed to a degree almost proportional to their distance from the "isometabolic point."

The sudden changes in the hydrogen ion concentration of the cultures, manifested by an increase of the pH value at the later part of the growth of the organism, were due to the autolysis of the mycelium, the products of autolysis being ammonia or other alkaline reacting substances—not different from those obtained generally from protein hydrolysis.

The nature of the reactions produced by the organism in potato starch and pectin followed the same order of behavior observed in dextrose solutions, *e.g.*, the initial pH value of certain cultures was either increased or decreased or remained constant. There is, however, a difference in the position occupied by the "isometabolic point" of the two different substances. The position occupied by the "isometabolic point" of potato starch lies near or at pH 5.2 and that of pectin near or at pH 6.5. The difference in the position of this point is possibly due either to a certain difference in the reaction of the metabolic products of the different sugars, or to a difference in the amount of sugars and other substances released during the hydrolysis of potato starch and pectin. Morever, the enzymes released by the organism for the hydrolysis of pectin and potato starch may interfere and even modify the extent of the changes in the initial hydrogen ion concentration of the culture solution and the position of the "isometabolic point."

BEHAVIOR IN GLUCOSIDES

For the study of the behavior of *F. cromyophthoron* in glucosides amygdalin was used. The molecular constitution of this substance is known as well as the products of its hydrolysis. The substances obtained when amygdalin is acted upon by emulsion are glucose, benzaldehyde, and hydrocyanic acid. In spite of the poisonous properties of hydrocyanic acid, the fungus could thrive and reproduce normally. Qualitative tests proved that the organism utilizes amygdalin in the hydrolysed form; all of the three products of hydrolysis by emulsin were found to exist in cultures in which the organism was left in contact with amygdalin for a certain length of time.

The changes produced in the initial H-ion concentration of various cultures by the action of *F. cromyophthoron* on amygdalin are recorded in table 4.

TABLE 4.—*Changes produced in the initial pH of a 1 per cent solution of amygdalin by F. cromyophthoron, from October 15 to November 15, 1923*

Date 1923	Culture solutions of a different initial pH pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0
10/15	3.0	4.0	5.0	6.0	7.0	8.0
10/20	3.5	4.5	5.0	5.8	6.5	7.4
10/25	4.5	5.0	5.0	5.2	6.0	6.6
10/30	5.0	5.0	5.2	5.0	5.2	5.5
11/5	5.0	5.2	5.2	5.0	5.0	5.2
11/10	5.2	5.4	5.4	5.2	5.2	5.4
11/15	5.4	5.6	5.8	5.4	5.6	5.8

Explanation on the Behavior in Glucosides

F. cromyophthoron is capable of changing the reaction of amygdalin solutions of different initial pH by either increasing or decreasing the H-ion concentration, the nature of the reaction and the extent of the changes depending on the initial pH of the particular culture. In cultures between pH 3.0 and 5.0, the reaction was changed by the organism by decreasing the H-ion concentration, in those between pH 5.0 and 8.0, by increasing it. The changes produced by the organism in the culture with initial pH 5.0 were practically nil. The behavior of the organism toward this pH value, *e.g.*, pH 5.0, in amygdalin solutions is comparable to that already observed in dextrose solutions at pH 3.8. Therefore, the "isometabolic point" of amygdalin is at or near pH 5.0.

It has been ascertained by chemical tests that the organism utilizes amygdalin in the hydrolysed form. It is not known, however, whether, besides glucose, hydrocyanic acid and benzaldehyde are utilized as they are produced or changed into some other form. If we assume, for the sake of argument, that benzaldehyde and hydrocyanic acid are not utilized, then two things ought to result: first, an accumulation of hydrocyanic acid and benzaldehyde in great quantities, and second, a reaction in the solution not differing from that of dextrose solutions in table 1, *e.g.* the isometabolic point of amygdalin ought to be near pH 3.8 and not at pH 5.0, if dextrose *alone* was assimilated. With an accumulation of great quantities of hydrocyanic acid, it is possible that the life of the organism may be endangered. As none of these reactions have been observed to take place, it is possible that both hydrocyanic acid and benzaldehyde are utilized to a certain extent in some form, and influence in this way the reaction of the solution. There are a number of changes which benzaldehyde may suffer, as well as hydrocyanic acid, which may render them available to the organism. Benzaldehyde, for example, may undergo auto-oxidation, add on hydrocyanic acid and hydrogen, or be changed to *hydrobenzamide* $((C_6H_5CH)3N_2)$ by the union of three molecules of benzaldehyde and two molecules of ammonia (6).

BEHAVIOR IN PROTEINS

Peptone, a protein derivative, was employed for the study of the behavior of *F. cromyophthoron* in protein solutions. The molecular constitution of peptone is not known. The resulting products of peptone hydrolysis are amino acids. The amino acids are substances containing one or more amino-groups in direct union with carbon and are both basic and acidic simultaneously, because of the reactivity of the amino and carboxyl radicals.

The manner in which proteins are assimilated by fungi is not well understood. Proteins, before they become assimilable, are hydrolysed into amino acids and may be utilized as such in certain cases, or broken into simpler compounds. It is known that certain bacteria under anaerobic conditions have the power of splitting off the amino group whereas under aerobic conditions they split off the carboxyl group. In higher animals, proteins are hydrolysed into amino-acids and used as such. The question therefore arises: is the amino-acid molecule taken into the fungal cell as it is, or is it broken into its simpler compounds, fatty acids, ammonia, or other different substances before it is assimilated? As the reactions of the organism show, in table 5, this constitutes a very important question, because it is related with the resistance of the protoplasm in high concentrations of hydroxyl ions.

The reactions produced by *F. cromyophthoron* in peptone solutions are recorded in table 5.

TABLE 5.—*Changes produced in the initial pH of 1 per cent peptone solution by F. cromyophthoron, from October 15 to November 15, 1923*

	Behavior in culture solutions of different initial pH					
Date 1923	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0
10/15	3.0	4.0	5.0	6.0	7.0	8.0
10/20	4.5	4.8	6.0	7.0	7.4	7.8
10/25	6.0	6.4	7.0	7.4	7.6	8.0
10/30	6.6	7.2	7.4	7.6	8.0	8.2
11/5	7.2	7.6	7.8	8.0	8.2	......
11/10	7.4	7.8	8.0	8.2	8.4	......
11/15	7.6	7.8	8.2	8.4	8.6	......

Explanations on the Behavior in Proteins

The behavior of *F. cromyophthoron* in peptone solutions at different initial pH, as it appears in table 5, indicates that the metabolic products decreased the hydrogen-ion concentration practically of every culture, regardless of its initial pH value. Chemical tests proved that the organism liberated ammonia in the solution during the assimilation of peptone. It is evident, therefore, that the decrease observed in the hydrogen-ion concentration of the different cultures was produced by the hydroxyl ions, resulting from the reaction of ammonia with water. The liberated ammonia was undoubtedly derived from the various amino-acids obtained from peptone by hydrolysis.

The nature of the reactions produced in peptone solutions by the above organism differs essentially in principle from that already observed in both dextrose and amygdalin solutions. No peptone solution ever showed properties such as have been observed in dextrose solutions at pH 3.8 and amygdalin solutions at pH 5.0. In other words, peptone solutions were one-direction reactions, *viz.*, toward increased alkalinity, and not amphoteric, as was the case with dextrose and amygdalin. The slight increase in the hydrogen-ion concentration; appearing at pH 8.0, was possibly due to the influence of the HCO_3 ions, resulting from the reaction of CO_2 with water.

The growth in mycelium development attained by the organism in peptone solutions at pH 6.0 and 7.0 was meager, and almost insignificant at pH 8.0, compared to that at pH 3.0, 4.0, and 5.0. The difference in the growth of the organism is undoubtedly due to the toxicity exerted by higher concentrations of hydroxyl ions in the cultures at initial pH 6.0, 7.0 and 8.0. High concentrations of hydroxyl ions may either flocculate the protoplasm of the organism or increase the permeability of the cell membrane (11). The inability of the organism to establish an "isometabolic point," it may not be strange to suppose, was due to the toxicity of high concentrations of hydroxyl ions, increasing in proportion with the decomposition of amino acids.

BEHAVIOR IN VEGETABLE AND ANIMAL DECOCTIONS

The studies on the changes produced in the initial pH of animal and vegetable decoctions by *F. cromyophthoron* were made by using beef extract and onion decoction. Both these substances are composed of mixtures of various properties of carbohydrates, glucosides, and proteins. The reactions produced in the medium as a result of the hydrolysis of these substances may influence the nature of the changes in the initial pH of the culture solution to an extent depending on the ratio of carbohydrates to proteins available in the media.

The changes produced by *F. cromyophthoron* in the initial pH of culture solutions of beef extract and onion decoction are recorded in tables 6 and 7.

TABLE 6.—*Changes produced in the initial pH of a 1 per cent beef extract solution by F. cromyophthoron, from October 15 to November 15, 1923*

Date 1923	Culture solutions of different initial pH pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0
10/15	3.0	4.0	5.0	6.0	7.0	8.0
10/20	4.0	5.0	5.5	6.5	7.0	7.8
10/25	6.0	6.8	7.0	7.2	7.2	7.4
10/30	7.0	7.2	7.2	7.2	7.3	7.5
11/5	7.2	7.2	7.2	7.4	7.4	7.7
11/10	7.2	7.4	7.4	7.6	7.6	8.0
11/15	7.4	7.5	7.6	7.8	8.0	8.2

TABLE 7.—*Changes produced in the initial pH of a 20 per cent onion decoction by F. cromyophthoron, from October 15 to November 15, 1923*

Date 1923	Culture solutions of different initial pH pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0
10/15	3.0	4.0	5.0	6.0	7.0	8.0
10/20	3.5	4.2	4.8	5.5	6.5	7.5
10/25	4.0	4.4	5.0	5.5	6.0	7.2
10/30	4.2	4.5	5.0	5.2	5.8	6.0
11/5	4.4	4.7	5.1	5.1	5.5	5.8
11/10	4.6	5.0	5.3	5.7	6.2	6.4
11/15	4.8	5.0	5.8	6.0	6.4	6.8

Explanation on the Behavior in Decoctions

The behavior of *F. cromyophthoron* in relation to the development of changes in the initial pH value of beef extract solutions and onion decoctions differed in the two cases. The organism changed but slightly the initial reaction of beef extract solutions at pH 7.2 and onion decoctions at pH 5.0; it produced, however, considerable changes in the remainder of the cultures of both media whose position on the pH scale was above or below the values mentioned. It follows, therefore, that the values pH 7.2 and 5.0 represent the "isometabolic point" of the two respective nutrient substances. The difference in the position of the "isometabolic point" on the scale of the pH values is possibly due to a corresponding difference in proportion of available carbohydrates, glucosides and proteins contained in the two different substances. The proportion of available carbohydrates to proteins is greater in the onion decoctions and smaller in the beef extract solutions. The ratio, therefore, of carbohydrates to proteins is the determining factor for the quantities of organic acids and alkaline reacting substances formed in the culture solution which correspondingly influence the position of the isometabolic point.

The slight acidity produced in the culture solution at pH 5.0, in table 7, at the early part of the growth of the organism was possibly due to the bicarbonate ions resulting from the reaction of CO_2 with water. The reactions produced in the remaining cultures, in tables 6 and 7, during the later growth of the organism, show a gradual decrease in the hydrogen-ion concentration. The nature of the reactions formed in the culture media during this period is due to the autolysis of the mycelium of the fungus and not to the reaction of the (proper) metabolic products.

The nature and extent of the reactions produced by organisms in culture media depend (a) on the chemical composition of the nutrient substance, and (b) on the initial reaction (pH value) of the particular culture.

DISCUSSION ON THE "ISOMETABOLIC POINT" OF NUTRIENT SUBSTANCES

The term "isometabolic point" is introduced in this presentation for the purpose of naming that initial reaction of culture solutions of any nutrient substance which, during the metabolic activities of an organism, is not altered in the concentration of the hydrogen or hydroxyl ions to any great extent by the reaction of the released metabolic products. It designates, in other words, that initial hydrogen-ion concentration of the culture solution of a nutrient substance which may or may not be changed slightly during the growth of an organism by the reaction of its metabolic products. The initial pH value of the cultures (of the same nutrient substance) whose position on the scale of pH values lies on either side of the "isometabolic point" is changed by the reaction of the metabolic products toward that of the "isometabolic point." This is accomplished by the reactions of the organism with the culture media which, in those cultures on the alkaline side of the "isometabolic point," tend to increase the hydrogen-ion concentration and decrease it in those on the acid side. The reactions, in this respect, however, are not controlled by the wilful operations of the organism, but by the chemical nature of the released metabolic products and possibly by other unknown biochemical factors. It must be kept in mind that the reaction at the "isometabolic point" is only maintained constant so long as some of the nutrient substance lasts in the culture medium. With the removal of the very last portion of this substance from the culture solution by the organism, the reaction changes suddenly toward alkalinity, due either to the development of reverse reactions in the metabolic products, or to the setting in of autolysis in the mycelium of the fungus.

The reactions produced in the culture solutions of the different nutrient substances, in tables 1 to 7, as a result of the growth of *F. cromyophthoron*, indicate that at certain initial pH values the organism can utilize many of these substances without disturbing the initial reaction either by an increase or decrease of the hydrogen ion concentration. The behavior of the organism, in this respect, tends to prove that there is some connection between certain hydrogen ion concentrations and the utilization of certain nutrient substances, the exact nature of which is baffling. It may be that at the "isometabolic point" the reaction is at equilibrium, *e.g.*, the organic acids and organic bases are formed in equivalent quantities in the culture solution and neutralized just as fast as they are liberated. Or it may be due to the buffer reaction of the culture solution in each particular case. The ions mainly responsible for the development of the reactions, which increased or decreased the hydrogen-ion concentration of the different culture solutions or maintained it constant, were those of the organic and, to a certain extent, inorganic acids, on the one hand, and the CO_3 and NH_4 ions, on the other hand. The acids, for instance, increased the hydrogen-ion concentration of the dextrose solution of the cultures at pH 8.0, 7.0, 6.0 and 5.0 toward pH 3.8, in table 1, and the CO_3 and NH_4 ions decreased the same in the cultures at pH 3.0 toward pH 3.8. The extent of the acids and alkalies formed in the culture media is controlled by the organism. The organism can grow in culture media at hydrogen-ion concentrations as high as pH 2.0 and as low as pH 10.0. Neither acids nor alkalies, formed in the culture media by the reactions of the organism, ever reached the above two extreme hydrogen-ion concentrations. The point, therefore, which is indicated as "isometabolic point" represents a hydrogen-ion concentration connected with certain nutritional or metabolic phases and not with the critical or death point of the organism.

The amphoteric nature of the reaction produced during the utilization of different nutrient substances by *F. cromyophthoron* suggests, to an analogous degree, the amphoteric nature of proteins (8). It is possible to make comparisons of the reactions of the isoelectric point of proteins and of the isometabolic point of substances, in certain phases. The studies of Robbins (13, 14) on the behavior of vegetable tissues at different hydrogen-ion concentrations indicate that the reaction of these tissues was amphoteric and isoelectric at certain pH values. Robbins's observations are very interesting and deserve a more extensive study.

The factors which, in general, seem to be responsible for the position which the "isometabolic point" occupies in the scale of pH values, during the utilization of a nutrient substance by an organism, are the following: (1) the ratio of available carbohydrates to proteins contained in a nutrient substance, (2) the resistance of the organism to the initial hydrogen or hydroxyl ion concentrations of the culture media, and (3) the resistance of the organism to the various products of hydrolysis or of metabolism which might be high concentrations of either hydrogen or hydroxyl ions, or other deleterious substances such as hydrocyanic acid, certain aldehydes and alcohols. The following citations explain the operation of the above factors in a number of cases. Harter and Weimer (4) report, in their studies on the behavior of *Rhizopus tritici* in various vegetable decoctions, that the initial reaction of prune, carrot, turnip and sweet potato decoctions was changed from less acid to more acid; and in Irish potato and string bean decoction, from acid to alkali. If we take for criterion the chemical composition of the tissues which were employed for the preparation of the different decoctions in order to explain the nature of certain of the reactions formed, we find that the prune, carrot, turnip and sweet potato decoctions contain more available carbohydrates, according to Wehmer (19), than the Irish potato and string bean decoctions. It is clearly seen that the increase which resulted in the initial hydrogen-ion concentration of the cultures of the former decoctions, and the decrease in the same of the latter were due to the difference in the chemical composition of the tissues. Paine and Chaudhuri (12) report, in their studies on the behavior of *B. solanisaprus* and *B. atrosepticus* in potato decoction, that the former organism changed the reaction of the decoction from pH 6.4 to 4.8 and of the latter from 6.4 to 7.2. It is interesting to consider, in this connection, also certain other reactions produced by both organisms on potato. From these it becomes evident that *B. atrosepticus* utilized the proteins of the potato for nutritional purposes and *B. solanisaprus* the carbohydrates. The difference in the behavior of the two organisms in the same nutrient substance was due to the reaction of the metabolic products resulting from the differential nutritional requirements of the organisms. The utilization of the carbohydrates of the potato by one of the organisms and of the proteins by the other constitutes a question not depending on the wilful preference of the organism for either of the substances, but on other physiological causes. It might be a question of susceptibility to certain hydrogen-ion concentrations resulting from either of the substances mentioned during the metabolic activities of the organism, or of inability to release the proper enzyme for the hydrolysis of the nutrient substance. Rosen (15) reports as follows on the behavior of *B. alboprecipitans* in culture media at different hydrogen-ion concentrations: (a) that his organism grew well in culture media at pH 6.6, 7.2, 7.4, and 7.6, but not at pH 4.8, 5.0, 8.5, 8.6, and 9.8, (b) that in carbohydrates the growth was meager in comparison to that obtained in beef broth, and (c) that the organism was unable to utilize carbohydrates, viz., various simple sugars, when furnished alone, but was able to do so when the sugars were mixed with beef broth. The behavior of *B. alboprecipitans,* in the first case, was due to the susceptibility to hydrogen ion concentrations higher than pH 5.0 and lower than pH 8.5. In the second case, the organism was unable to utilize the different simple sugars and produce an appreciable growth, because the resulting concentrations of hydrogen ions were sufficiently high to inhibit the growth of the organism. The profuse mycelial growth attained by the organism in beef broth was due to the chemical composition of this substance, the quantity of available carbohydrates to proteins being of such a proportion as to permit the organism to utilize the substance completely without producing changes in the initial reaction toxic to the welfare of the organism. In the third case, the ability of the organism to utilize sugars when mixed with beef broth was due to the favorable hydrogen-ion concentrations resulting from the reaction (probably neutralization) of oppositely charged metabolic products released from the two different nutrient substances.

The reactions produced in the surrounding solution by the metabolic products of micro-organisms from different nutrient substances, considered together with the susceptibility of various microorganisms to certain hydrogen-ion concentrations, may constitute a factor of considerable importance for the initiation or inhibition of many diseases. The behavior of *F. cromyophthoron* and of the organisms mentioned in the preceding paragraph is of interest not alone to the plant physiologist but to the plant pathologist as well. The different reactions produced by parasites during the utilization of host tissues may be employed by plant pathologists to obtain some information on the methods of the parasite and to understand, to a certain extent, the nature of the disease. With organisms very sensitive to the slight changes of hydrogen-ion concentrations, it is easy to see how the resulting reactions in the surrounding solution may influence the initiation or inhibition of a disease. The sources from which appropriate hydrogen-ion concentrations may be released, for the initiation or inhibition of various fungal and bacterial diseases, are: (a) the tissues of the host, and (b) the soil solution. The former may furnish a number of hydrogen-ion concentrations due to fluctuations in the sap acidity and in the composition of the tissues, as far as the ratio of carbohydrates to proteins is concerned, at different seasons of the year and different stages of growth. The latter, in an analogous manner, may furnish a number of hydrogen-ion concentrations, due either to reactions in the mineral constituents of the soil solution or to biological reactions.

SUMMARY

Fusarium cromyophthoron, grown in culture media of different nutrient substances at different initial hydrogen-ion concentrations, formed substances (metabolic products) which, in certain cases, maintained constant the initial reaction of the culture solution and, in others, either increased or decreased the hydrogen-ion concentration. In dextrose solutions, the hydrogen-ion concentration was increased in the cultures at initial pH 4.0, 5.0, 6.0, 7.0, and 8.0, and decreased at pH 3.0; the final reaction in the majority of the cultures pointing toward pH 3.8. In amygdalin solutions, the hydrogen-ion concentration was increased in the cultures at pH 6.0, 7.0, and 8.0, and decreased at pH 3.0 and 4.0; the final reaction in this substance pointing toward pH 5.0. In peptone solutions, the hydrogen-ion concentration was decreased practically in every culture, viz., at pH 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0. In potato starch solutions, the hydrogen-ion concentration was increased in the cultures at pH 6.0, 7.0, and 8.0, and decreased at pH 3.0, 4.0, and 5.0; the final reaction pointing toward pH 5.2. In pectin solutions, the hydrogen-ion concentration was increased in the cultures at pH 7.0 and 8.0, and decreased at pH 3.0, 4.0, 5.0, and 6.0; the final reaction pointing toward pH 6.4. In beef broth, the hydrogen-ion concentration was increased in the cultures at pH 8.0, and decreased at pH 3.0, 4.0, 5.0, 6.0, and 7.0; the final reaction pointing toward pH 7.4.

To the particular hydrogen-ion concentration toward which the final reaction was pointing, in the cultures of the different nutrient substances, the term "isometabolic point" has been assigned. Therefore, the "isometabolic point" of dextrose solutions lies near or at pH 3.8, that of amygdaline at pH 5.0, of potato starch at pH 5.2, of pectin at pH 6.4, and of beef broth at pH 7.4. The initial reaction of the cultures at the pH value of the "isometabolic point" was maintained more or less constant during the growth of the organism.

The reactions of the metabolic products, formed during the growth of microorganisms on different vegetable tissues and particularly host tissues, may be profitably employed by the plant pathologists to obtain some information on the methods of parasites in the development of diseases.

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A PRELIMINARY STUDY OF FUNGOUS ACTION AS THE CAUSE OF DOWN CORN

L. W. DURRELL[1]

WITH FOUR FIGURES IN THE TEXT

The breaking of corn stalks in the fall before the crop is harvested, as shown in figure 1, not only results in considerable financial loss due to rot-

FIG. 1. Broken plants in this field resulted from the fungous invasion of the nodes, coupled with a high wind.

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[1] The writer wishes to acknowledge the assistance of Dr. I. E. Melhus, Pathologist, Iowa State Agricultural College, for helpful suggestions in the work and in preparation of the manuscript; and also valuable suggestions offered by J. Pinsky, Colorado Agricultural College.

ting of the down ears, but also increases the cost and inconvenience of harvesting. The cause of this breaking has been attributed to a wide range of agencies by the corn growers, such as high wind, so-called "running out" of varieties, soil depletion, micro-organisms, etc. The lack of proof as to the exact cause of the breaking, coupled with observations extending over a period of seven years, wherein many of the broken stalks were found badly disorganized and frequently infected with micro-organisms, suggested the possibility that the rigidity or stiffness of the stalks had been partially destroyed by certain fungi that were prevalent on the nodes, *i.e., Diplodia zeae, Gibberella saubinetti, Basisporium gallarum,* and *Fusarium* sp. In order to determine if the invasion by these fungi results in a weakening of the nodes, and why the breaking occurs four or five nodes above the ground, the following experiments were planned and carried out during 1922 and 1923.

METHOD OF TESTING BREAKING STRENGTH OF STALKS

The tests of the strength of healthy and infected nodes of cornstalks were made by using a lever as shown in fig. 2. The cornstalks to be broken were placed on two blocks six inches apart with the node in the center. The distance of six inches was arbitrarily chosen as it was a convenient length and fitted the distance between the centers of most internodes. The lever was forced down upon the node until the stalk broke, a record being made of the pressure exerted on a spring balance. As the lever had a six to one ratio, this pressure was multiplied by six to get the weight necessary to break the stalk. The stalks used were mostly of the variety Iodent grown on the Iowa Experiment Station Farm in 1923. The stalks were taken

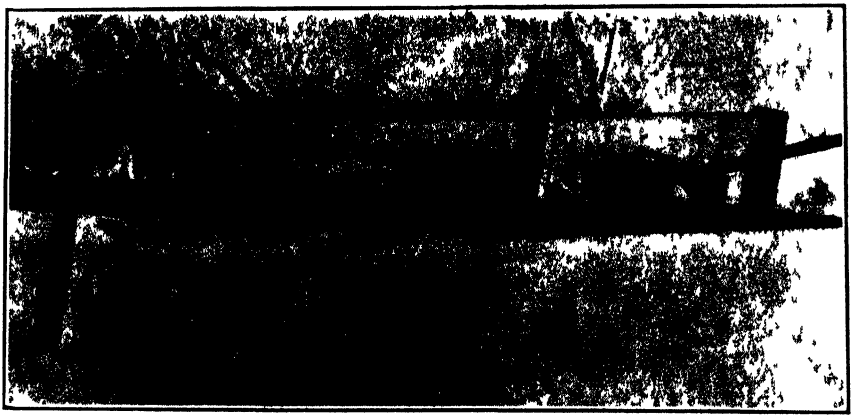

FIG. 2. Lever used in testing breaking strength of stalks.

directly from this field to the laboratory and broken. The fungus flora was determined by microscopic examination and pure cultural methods after the breaking strength had been determined.

THE BREAKING STRENGTH AND FUNGOUS FLORA OF STALKS IN 1923

Using the above described apparatus, 61 stalks of corn were broken. The stalks were gathered during the season 1923, beginning just before flowering. Uninfected material was obtained at this time. Later in the season infected material was tested. In the following table are given the results of these breaking tests together with the fungous flora of the different infected nodes:

TABLE 1.—*Strength of corn nodes and fungi found in them*

No. of stalk	Node 1		Node 2		Node 3		Node 4		Node 5	
	Fungus flora	Breaking strength pounds	Fungus flora	Breaking strength pounds	Fungus flora	Breaking strength pounds	Fungus flora	Breaking strength pounds	Fungus flora	Breaking strength pounds
1	B	30	D	48	F	18	F	18	F	42
2		150				180		150		
3		198		180		90				
4		180	F	137	D	90	D	60		
										30
6	G	48	G	48	G	36				
										6
8	G	18	G	30	D	30		18		
9	G	54	G	36	D	30	D	24		12
10				126				78		
11	F	60	D	30	F	42				
12	D	30	D	60	G	54	D	36		
13	G	72	G	54	G	48	G	30		
14	G	66	D	84	G	78				
15	H	78	G	150		90	G	84		
16	G	78	G	66	G	90	G	60	G	54
17	G	30	G	36	G	30	G	36	D	18
18			G	6	G	24	G	30		30
19			G	30	G	30	G	30		
20	F	84	D	48	G	36	D	42		
21			G	6	G	18	G	12		
										24
23			G	132	B	114	D	120		120
24			D	54	D	54	D	36	D	18
25				60		54		49		30
26			D	276	D	210	D	156		156
27		156		192		162		120		72

TABLE 1 (*Continued*).—*Strength of corn nodes and fungi found in them*

No. of stalk	Node 1		Node 2		Node 3		Node 4		Node 5	
	Fungus flora	Breaking strength pounds	Fungus flora	Breaking strength pounds	Fungus flora	Breaking strength pounds	Fungus flora	Breaking strength pounds	Fungus flora	Breaking strength pounds
28				276		216		168		144
29				132		90				66
30				306				204		108
31				60		36		24		42
32						120		96		60
33		90	F	144				96		72
34		90	F	126				96		
35	F	90		66		84		36		
36				120		84		48		48
37				144				90		
38	G	126			G	72		60		
39	F	30				36		36		
40		84		72						
41		330		216				56		
42		306		138						108
43		186		150		90				
44				132		96		84		
45		162				132		102		
46		252				132				
47		132		102				66		
48		234				138		126		102
49		168				102				
50		120		90		66				42
51		144				96				
52		174				144		90		
53		114				84				53
54		126				84		54		
55		102		84		60		48		
56				174		174		150		108
57		150		120		72				18
58				66		54		36		12
59		162				132				96
60		120				90		84		48
61		150				132		84		78

D—*Diplodia zeae*
G—*Gibberella saubinettii*
B—*Basisporium gallarum*
F—*Fusarium* sp.

Of the 213 nodes broken, 82 were infected with one of the following fungi: *Diplodia zeae, Gibberella saubinetti, Basisporium gallarum,* or *Fusarium* sp. It is important to note that in the case of infected stalks infection most frequently occurs at the second, third and fourth nodes. The cause of the frequent infection of these nodes has been described and explained by the author (1, 2)as due to the earlier loosening of the leaf sheath of the lower leaves from the stalk, permitting the inoculum to gain entrance, and to the greater moisture near the ground favoring infection. The general effect of these organisms on the tissues at the nodes is shown in figure 3.

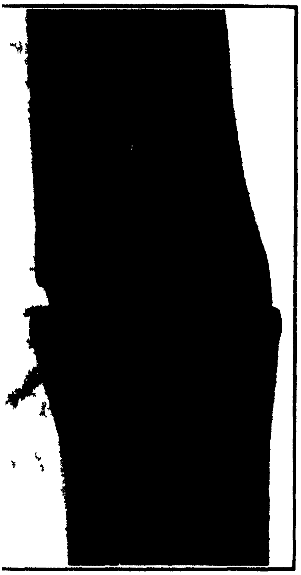

FIG. 3. Corn node split to show disintegration caused by fungous attack.

The breaking strength of the 213 nodes tested ranged from a minimum of 6 pounds to a maximum of 330 pounds under the condition of the experiment. It is quite significant that the strength of the infected nodes is much lower than that of the uninfected ones. This relation of strength to infection is summarized in table 2.

TABLE 2.—*Pressure at which infected and uninfected nodes break*

	Node 1	Node 2	Node 3	Node 4	Node 5
Average of infected nodes	55	72	54	47	33
Average of uninfected nodes ..	163	133	104	84	64

In the above table it may be noted that the infected nodes have lost about one-half their strength, also that there is a gradual decrease in strength in progressing from the lower to the upper nodes. The upper nodes, however, are not subjected to the leverage of the wind that the lower part of the stalk must bear.

Histological study of the stalks reveals a greater degree of lignification in the lower nodes as illustrated in fig. 4. At first only the vascular bundles of the stem are lignified, but as the plant increases in age this lignification extends to the cellulose of the pith cells surrounding these until a thick sclerenchymatous area is formed on the outer periphery of the stalk. This extra lignification constitutes a very considerable layer up to the third and fourth nodes. Holbert and Koehler (3) have described a somewhat similar condition in the roots of different strains of corn in their relation to anchorage.

DISCUSSION

In tests of the nutritional relations of *Diplodia zeae, Gibberella saubinetti* and *Basisporium gallarum,* it was found that these organisms are capable of assimilating cellulose, and to a slight extent, lignin. Microchemical tests made on infected nodes also show the destruction of not only the cellulose connective tissue, but also the lignified fibers. This is in a degree comparable to the well-known effect of wood-destroying fungi whose solvent action on cellulose and lignin results in shrinkage and cracking of wood.

The disintegration of the tissues of the stalk (Fig. 3) results in ready breaking under strains that would be easily withstood by healthy stalks. Thus, high wind results in down corn after the nodal infection has progressed enough to weaken the stalks. In some fields this is very obvious for all the broken stalks point in the same general direction (Fig. 1). Pammel, King and Seal (4 and 5) found this condition in Iowa corn fields in 1914, but did not measure the effect or record the extent of nodal infection.

It is interesting in this connection to correlate the relation of wind velocity and pressure to the breaking strength of the stalks.

Taking, for example, the maximum velocity for September, 1923, 40 miles per hour, the given pressure per square foot amounts to 7.8 lbs. Further, for example, take an average cornstalk 10 ft. tall, measuring 1.25 inches in diameter at the base and .37 inches at the top. Such stalk would expose 97.2 sq. inches of surface to the wind, irrespective of leaves and ears. However, in September the leaves are largely torn away or badly frayed, making their resistance difficult to determine, so the stalk only will be considered. On the basis of a pressure of 7.8 pounds per sq. foot, a 10-foot stalk will receive 5.2 pounds of pressure distribution over its length.

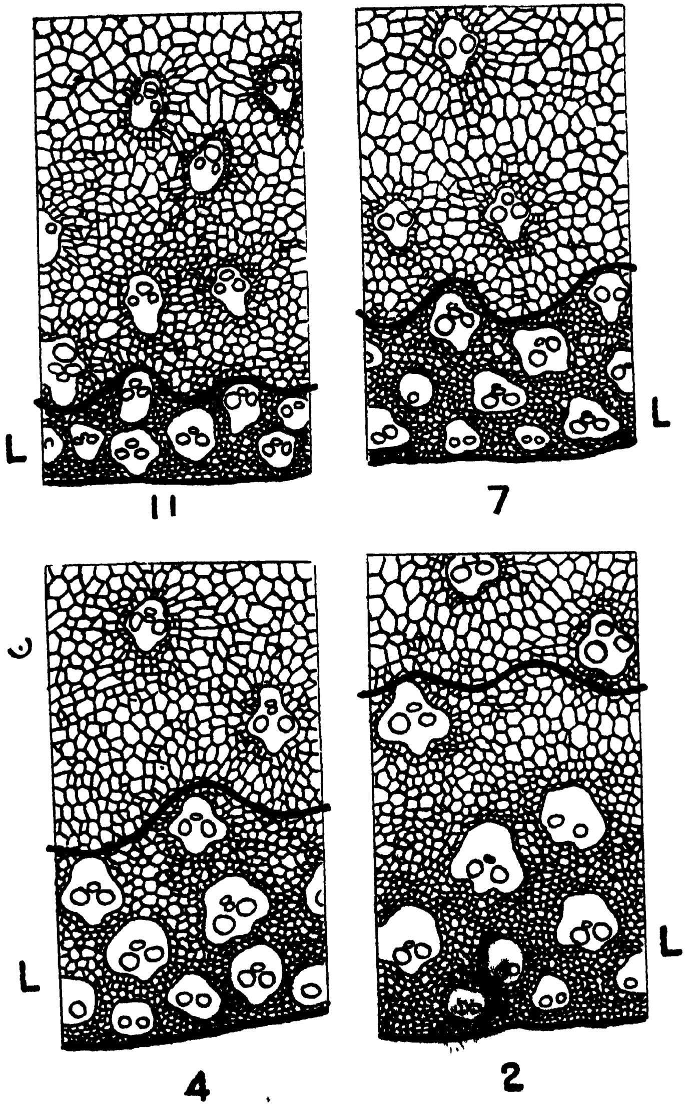

FIG. 4. Sections of corn stalk near second, fourth, seventh and eleventh nodes, showing the greater amount of lignification (L) at the lower nodes.

In determining the maximum stress induced in a section of a tapering cylinder, such as a cornstalk, the following formula might be used:

$$S' = \frac{32W\,(L - X)^2(d - KX)}{2\pi L\,((d - KX)^4 - (d' - K'X)^4)}$$

S' — stress produced by wind
L — total length of stalk in inches
d — outer diameter of base in inches
d' — inside diameter of base in inches
X — distance in inches of any section from base of stalk
K — decrease of outer diameter per inch in length
K — decrease of inner diameter per inch in length
W — total pressure due to wind
W' — breaking load determined by breaking machine
L' — distance between supports in breaking machine
S — stress produced in breaking machine.

The maximum stress, S, as determined by breaking the cornstalk in the above described machine, may be expressed in general by the formula

$$S = \frac{32W'L'd'}{\pi\,(d^4 - d'^4)}$$

With the use of these formulas, substituting known values from a representative stalk, it may be shown that it requires a 68-mile wind to break a healthy first node, while a 33-mile wind will break the average infected node.

Healthy third, fourth and fith nodes will be broken by 55-, 50-, and 34-mile winds, respectively, although the average infected third, fourth, and fifth nodes will break under 39-, 37-, and 24-mile winds, where the elasticity of the tissues is destroyed by fungi.

Under field conditions, as before stated, the leaves and ear furnish additional resistance, which would increase chances of breaking of infected nodes.

It might be argued that under such pressure all the tops of the stalks would break. It is obvious the same leverage is not exerted on the tops as on the lower parts of the stalk. Then, too, the topmost nodes are seldom infected, and so retain their elasticity. As shown by the writer (*l.c.*) in the case of *Diplodia zeae,* infection is most prevalent at the lower nodes because of the moister conditions nearer the ground and because the leaf sheaths first open there, allowing the entrance of organisms. Were it not for the greater lignification of the lowest nodes and the fact that the fungi under consideration attack lignin less readily than cellulose, these nodes would break more commonly than those a little higher up as they receive the greatest strain.

SUMMARY

It has been observed during the past five years that in down corn the stalks are frequently broken at the fourth node or above.

Diplodia zeae, Gibberella saubinetti, Basisporium gallarum, and *Fusarium* sp. are prevalent on the broken stalks and weaken them by partially destroying the tissues at the nodes.

Infected nodes are not as strong as healthy ones according to the breaking tests recorded. A correlation exists between the breaking of the weakened nodes and the wind pressure exerted on the stalk.

The lower nodes are stronger than the upper ones, due to the greater lignification, which explains why the stalk more often breaks at or above the fourth node.

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ALIQUOT FUSARIA TROPICALIA NOVA VEL REVISA[1]

H. W. WOLLENWEBER[2] AND O. A. REINKING[3]

INTRODUCTION

During an investigation conducted by the junior author on Fusaria in relation to various banana diseases in Central America, a large number of different strains of Fusaria were collected and studied. The fungi described in the paper comprise a limited number of new organisms, and revised forms of older species, that were found, out of some two hundred and fifty strains studied. In a few instances it was deemed necessary to revise and enlarge the descriptions of various sections and species for which only a meager or indefinite diagnosis existed. The descriptions have all been based upon pure culture studies carried on during the past two years, at first by the junior author and finally as a joint study by both authors. In the latter study access was had to all strains of Fusaria at present available in the United States and Europe.

In a study of the relation of Fusaria to plant diseases it is important to obtain a complete knowledge of all Fusaria present on plants and then to determine their pathogenicity. The first step in this procedure has been undertaken in a study of Fusaria in relation to banana diseases and the present number of new organisms were obtained. Their parasitic nature has not been determined. The majority belong to sections that include parasitic species and it is highly probable that further studies will prove the pathogenicity of a number of these. This is particularly true of those in section Liseola. One species in this section (*F. moniliforme*) is a common plant pathogen producing the kernel mold disease of corn; while the others are prevalent in the tropics associated with living and dead plants, and some apparently attack living tissue. In section Martiella, *F. theobromae* has been reported as causing a rot of cacao pods.

The systematic arrangement followed in the paper is according to the grouping of Fusaria into sections as given in a former article.[4]

Pure cultures of each species identified have been placed in the Pathological Collections of the Bureau of Plant Industry, United States Department of Agriculture, and the Centraal-Bureau voor Schimmelcultures, Baarn, Holland.

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[1] Published as a contribution from the Agricultural Research Department, United Fruit Company, Boston, Mass.

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[4] Wollenweber, H. W., Sherbakoff, C. D., and Reinking, O. A., with the cooperation of Johann, Helen and Bailey, Alice A. Fundamentals for taxonomic studies of Fusarium. Jour. Agr. Research 30: No. 2. 1925.

DESCRIPTIONS OF NEW AND REVISED SECTIONS, SPECIES, VARIETIES AND FORMS

FUSARIUM Link

III. *Sectio Sporotrichiella* Wr.

Lewis, Charles E. 1913. Comparative studies of certain disease producing species of Fusarium. Maine Agr. Exp. Sta. Bul. 219, *p. 256.*

Sherbakoff, C. D. 1915. Fusaria of potatoes, N. Y. (Cornell) Agr. Exp. Sta. Mem. no. 6. *p. 183.*

Conidiis plerumque in aërium mycelium sparsis, pyriformibus vel globosis continuis, rarius septatis, sporodochiis deficientibus in speciebus typicis, macroconidiis raris, fusiformibus vel falcatis, septatis; chlamydosporis raris. Species: *F. poae* (Pck.) Wr., *F. sporotrichioides* Sherb., **F. chlamydosporum** n. sp.

Conidia for the most part scattered in the aerial mycelium, pyriform to globose, unicellular, sporodochia absent in typical species, macroconidia few, fusiform or sickle-shaped, septate; chlamydospores rare. Species *F. poae* (Pck.) Wr., *F. sporotrichioides* Sherb., **F. chlamydosporum** n. sp.

1. **Fusarium chlamydosporum** n. sp.

Microconidiis sparsis ad conidiophoros irregulariter ramosos dispositis, ovoideo-pyriformibus, plerumque continuis, 6–9 × 2.5–4.0 µ, raro 1-septatis, 11–16 × 3.0–3.5 µ; macroconidiis liberis, raris, falcatis, 1–3-septatis; 3-septatis, 27–32 × 3.5–4.5 µ; sporodochiis nullis; mycelio aerio floccoso, e roseo atropurpureo, e sulfureo ochraceo; stromate expanso vel erumpenti, tuberculari plectenchymico; chlamydosporis globosis vel pyriformibus, rugulosis, ochraceis, terminalibus vel intercalaribus, singulis, binis, catenulatis, acervalibus, 10–16 µ diam.

Habitat: Ad basim Musae sapientum (R38), humo aëreque, Honduras, Amer. centr. (O. A. Reinking no. R 38.)

Microconidia borne on irregularly branched conidiophores, ovoid, pyriform, mostly unicellular, 6–9 × 2.5–4.0 µ, rarely 1-septate, 11–16 × 3.0–3.5 µ; macroconidia scattered, rare, sickle-shaped, 1–3-septate; 3-septate, 27–32 × 3.5–4.5 µ; sporodochia not present; mycelium floccose from rose to carmine, from sulphuric to dark brown; plectenchymic stroma sometimes tubercular; chlamydospores globose to pear shaped, rugose to spiny, ochreous, terminal or intercalary, one to two celled, in chains or clusters, 10–16 µ in diameter.

Habitat: On the exterior of the pseudostem of a living banana plant (*Musa sapientum*) (R 38) also in the soil and in the air. Honduras, Central America. (O. A. Reinking no. R 38.)

Fusarium chlamydosporum n. sp. produces mostly microconidia of the sporotrichum type, a few sickle-shaped macroconidia and an abundance of large chlamydospores that are characteristic for the species. The aerial mycelium develops swellings, in some hyphae, that differ from chlamydospores in having no double wall. Such swellings are formed in *F. flocciferum* Cda. of the section Discolor, and in species of the section Gibbosum.

✓V. *Sectio Arthrosporiella* Sherb.

Sherbakoff, C. D. 1915. Fusaria of potatoes. N. Y. (Cornell) Agr. Exp. Sta. Mem. no. 6, *p. 161.*

Aërio mycelio abundante, albo-incarnato, sporodochiis et pionnote; microconidiis in aërio mycelio fusiformibus vel lanceolatis, apedicellatis, 0–3-septatis; macroconidiis in massis isabellinis vel ochraceis, falcatis, attenuatis, saepe pedicellatis, 3-pluriseptatis; chlamydosporis typicis intercalaribus, stromate ochraceo, castaneo, roseo, expanso, interdum nodoso acervali.

Aerial mycelium abundant, whitish to flesh color, sporodochia and pionnotes present; microconidia in aerial mycelium, spindle-shaped or lanceolate, apedicellate, 0–3-septate; macroconidia in masses, Isabella color or ochraceous, sickle-shaped, attenuate, often pedicellate, 3-more septate; chlamydospores typically intercalary, stroma ochraceous to chestnut-brown or rosy, spread out, sometimes sclerotially erumpent.

✓2. *Fusarium semitectum* Berk. et Rav.

Berkeley, J. M. 1875. Notices of North American fungi. Grevillea 3: *p. 98.*

Saccardo, P. A. 1886. Sylloge Fungorum 4: *p. 718.*

Wollenweber, H. W. 1917. Fusaria autographice delineata. Ann. Mycol. 15: *p. 11, fig. 112.*

Aërio mycelio albo-incarnato vel isabellino; stromate plectenchymico atro-ochraceo, interdum violaceo-carmineo; chlamydosporis intercalaribus; sporodochiis nullis; conidiis aërio mycelio instratis, fusiformibus, lanceolatis, subcurvatis, apedicellatis, appendiculatis, minoribus 0–2-septatis, maioribus 3–5–(6–7)-septatis; 0-septatis, 12 × 3.0–3.5 μ; 1-septatis, 11–21 × 2.5–4.5 μ; 2-septatis, 16–24 × 3.25–5.0 μ; 3-septatis, 18–40 × 3.0–5.5 μ; 4-septatis, 29–45 × 4.0–5.5 μ; 5-septatis, 36–52 × 4.0–5.5 μ; 6–7-septatis, 37–57 × 4.5–5.5 μ.

Habitat: Ad insertionem pistilli fructus putridi Lycopersici esculenti, in floribus emortuis, fructibus putridis, disco axis caesae Musae sapientum (R 50), in aëre, Honduras, Amer. centr. (O. A. Reinking no. R 50.)

Aerial mycelium white to flesh color or Isabella color, stroma plectenchymic, dark ochraceous sometimes violet-carmine; chlamydospores intercalary; sporodochia absent; conidia scattered in aerial mycelium, spindle-shaped, lanceolate, slightly curved, apedicellate, appendicular, when smaller 0–2-septate, when larger 3–5–(6–7)-septate; 0-septate, 12 × 3.0–3.5 μ; 1-septate, 11–21 × 2.5–4.5 μ; 2-septate, 16–24 × 3.25–5.0 μ; 3-septate, 18–40 × 3.0–5.5 μ; 4-septate, 29–45 × 4.0–5.5 μ; 5-septate, 36–52 × 4.0–5.5 μ; 6–7-septate, 37–57 × 4.5–5.5 μ.

Habitat: At blossom end rot of tomato (*Lycopersicum esculentum*), in dead floral parts, decaying fruit and interior of pseudostem of diseased banana plant (*Musa sapientum*) (R 50), and in the air. Honduras, Central America. (O. A. Reinking no, R 50.)

Fusarium semitectum Berk. et Rav. is generally widespread throughout banana plantations, growing on the dead floral remains at the end of the individual banana fruits and decaying banana fruit and floral parts on the ground.

3. Fusarium camptoceras n. sp.

Aërio mycelio albo-incarnato vel isabellino; stromate atro-ochraceo interdum incarnato; chlamydosporis intercalaribus; sporodochiis nullis; conidiis aërio mycelio instratis, falcatis, utrinque subacutis, apice plus minusve constricto, basi rotundata vel conica, apedicellata interdum vero appendiculata, minoribus 0–2-septatis, maioribus 3–5–(6–7)-septatis; 0-septatis, 7–12 × 2.5–3.5 μ; 1-septatis, 11–18 × 3.0–4.0 μ; 2-septatis, 14–26 × 3.5–5.0 μ; 3-septatis, 17–32 × 3.5–5.5 μ; 4-septatis, 22–40 × 3.5–6.0 μ; 5-septatis, 29–52 × 4.5–6.0 μ; 6-septatis, 25–58 × 4.5–5.5 μ; 7-septatis, 31–51 × 4.0–5.5 μ.

Habitat: In fructibus putridis Musae sapientum, Theobromae cacao (R 78), in humo, Honduras, Amer. centr. (O. A. Reinking no. R 78.)

Aerial mycelium white to flesh color or Isabella color; stroma dark ochraceous sometimes flesh color; chlamydospores intercalary; sporodochia absent; conidia scattered in the aerial mycelium, sickle-shaped, slightly pointed at ends, more or less constricted at top end, rounded or conical at base, sometimes apedicellate, however, appendicular, smaller conidia 0–2-septate, larger conidia 3–5–(6–7)-septate; 0-septate, 7–12 × 2.5–3.5 μ; 1-septate, 11–18 × 3.0–4.0 μ; 2-septate, 14–26 × 3.5–5.0 μ; 3-septate, 17–32 × 3.5–5.5 μ; 4-septate, 22–40 × 3.5–6.0 μ; 5-septate, 29–52 × 4.5–6.0 μ; 6-septate, 25–58 × 4.5–5.5 μ; 7-septate, 31–51 × 4.0–5.5 μ.

Habitat: On decaying fruit of banana (*Musa sapientum*) and cacao (*Theobroma cacao*) (R 78), and in the soil. Honduras, Central America. (O. A. Reinking no. R 78.)

VI. *Sectio Gibbosum* Wr.

Wollenweber, H. W. 1913. Studies on the Fusarium problem. Phytopathology, 3, *p. 31, fig 1, L and M.*

Conidiis in sporodochiis et in pionnote, pallido ochraceis vel aurantiacis, dorsiventralibus falcatis, elliptice, parabolice vel hyperbolice curvatis, utrinque attenuatis, pedicellatis; interdum conidiis minoribus aerio mycelio instratis "commae" forma, 0–3-septatis, utrinque rotundatis vel leniter constrictis, apedicellatis; chlamydosporis intercalaribus; sclerotiis rarioribus interdum atro-coeruleis; stromate plectenchymico ochraceo, badio vel carmineo.

Conidia in sporodochia and in pionnotes, pale ochraceous to orange, dorsiventral sickle-shaped, elliptical, parabolical of hyperbolical curved, attenuate at both ends, pedicellate; sometimes smaller conidia in aerial mycelium, "coma" shaped, 0-3-septate, rounded at both ends or slightly constricted, apedicellate; chlamydospores intercalary; sclerotia rare, sometimes dark blue; stroma plectenchymic, ochraceous, chestnut-brown or carmine.

4. *Fusarium bullatum* Sherb.

Sherbakoff, C. D. 1915. Fusaria of potatoes. N. Y. (Cornell) Agr. Exp. Sta. Mem. no. 6, *p. 198.*

Auctore Sherbakoff hic fungus sub sectione Ferruginosa Sherb. descriptus est: conidiis falcatis, pedicellatis plerumque 5-septatis, 42 × 4.3 (31–47 × 4.1–4.9) μ, cremeis vel salmoneis; chlamydosporis intercalaribus, singulis, catenulatis vel acervalibus; stromate ex hyalino pallide luteolo tincto.

This fungus has been described by Sherbakoff under his section of Ferruginosum with sickle shaped, pedicellate, mostly 5-septate, 42 × 4.3 (31–47 × 4.1–4.9) μ, cream or salmon color conidia; chlamydospores intercalary, in chains or clusters; stroma from hyaline to pale golden.

5. *Fusarium bullatum* Sherb. var **minus** n. v.

A typo differt conidiis minoribus; conidiis in sporodochiis et in pionnote, pedicellatis, plerumque 3-septatis, 21–40 × 3.0–4.0 μ rarius 4-5-septatis; 5-septatis, 33–42 × 3.5–4.5 μ; conidiis aerio mycelio instratis interdum subnormibus apedicellatis, 3(1–5)-septatis.

Habitat: In partibus putridis plantae ignotae in insula Jamaica (C. G. Hansford no. 14, R 233).

Differs from the type species by smaller conidia; conidia in sporodochia or in pionnotes, pedicellate, mostly 3-septate, 21–40 × 3.0–4.0 μ; seldom 4-5-septate; 5-septate, 33–42 × 3.5–4.5 μ; conidia in aerial mycelium sometimes subnormal apedicellate, 3(1–5)-septate.

Habitat: In plant debris. Jamaica (C. G. Hansford no. 14, R 233). The fungus was obtained through the courtesy of Mr. C. G. Hansford of Jamaica.

6. *Fusarium bullatum* Sherb. var. **brevius** n. var.

A typo differt conidiis brevioribus 5(3–5)-septatis; 5-septatis, 31–44 × 3.0–4.0 μ; 3-septatis, 21–36 × 2.5–3.5 μ.

Habitat: In ramis emortuis Hybisci rosae-sinensis, in foliis aliisque partibus Musae sapientum (R 47), Honduras, Amer. centr. (O. A. Reinking no. R 47.)

Differs from the type species by shorter conidia, 5(3–5)-septate; 5-septate, 31–44 × 3.0–4.0 μ; 3-septate, 21–36 × 2.5–3.5 μ.

Habitat: On dead hibiscus stems (*Hibiscus rosa-sinensis*), decaying banana leaves and plant parts (*Musa sapientum*) (R 47). Honduras, Central America. (O. A. Reinking no. R 47.)

7. **Fusarium longipes** n. sp.

Stromate expanso vel erumpente verrucosa, aerio mycelio ex albo carmineo et ochraceo; conidiis in sporodochiis et in pionnote, interdum in columnis dispositis, ochraceo-aurantiacis, elongatis falcatis, parabolice vel hyperbolice curvatis attenuatis, apice procero plus minusve voluto, basi distincte longipedicellata; 5(4–6)-septatis, 63–104 × 2.75–4.0 μ; conidiis minoribus aerio mycelio instratis brevipedicellatis; 5-septatis, 36–53 × 3.0–4.0 μ; chlamydosporis subverrucosis, intercalaribus, plerumque singulis, 6–9 μ diam.

Habitat: Ad folia matura viventia Musae sapientum, Honduras, Amer. centr. (O. A. Reinking no. R 34.)

Stroma spread out or verrucose erumpent, aerial mycelium from white to carmine and ochraceous; conidia in sporodochia and in pionnotes, sometimes in columns, ochraceous to orange, elongate sickle-shaped, parabolically or hyperbolically curved, attenuate with whip-like top end sometimes very much curved, footed basal cell long, 5(4–6)-septate, 63–104 × 2.75–4.0 μ; smaller conidia in aerial mycelium with a short footed base; 5-septate, 36–53 × 3.0–4.0 μ; chlamydospores sometimes spiney, subverrucose, intercalary, mostly singular, 6–9 μ diameter.

Habitat: On mature and living leaves of banana (*Musa sapientum*). Honduras, Central America. (O. A. Reinking no. R 34.)

VII. *Sectio Roseum* Wr.

Wollenweber, H. W. 1918. Conspectus analyticus Fusariorum. Berichte der Deut. Bot. Gessell. 35: *p. 739.*

8. *Fusarium anthophilum* (A. Br.) Wr.

(Syn. *Fusisporium anthophilum* A. Braun.)

Braun, A. 1875. In Rabenhorst Fungi Europaei. no. 1964.

Wollenweber, H. W. 1917. Fusaria autographice delineata. Ann. Mycol. 15: *p. 14, fig. 176–177.*

Stromate pallido, nunquam carmineo; conidiis liberis, in pionnote, rarius in sporodochiis, subulatis vel falcatis, Fusario herbarum similibus, pedicellatis, 3-5-septatis, 35–70 × 2.5–4.0 (30–82 × 2.5–4.5)μ; rarissime 6-pluri-septatis; conidiis liberis lanceolatis vel subcurvatis, utrinque attenuatis, apedicellatis vel appendiculatis; chlamydosporis nullis.

Habitat: In corollis et antheris Succisae pratensis, quae corollae clausae manent, mox arescunt et putrescunt, Berchtesgaden Bavaria in Germania (A. Braun). In inflorescentiis emortuis Citri aurantifoliae, foliis aridis Dauci carotae, pedunculis fructuum, foliis aliisque partibus emortuis Musae sapientum, legumine maturo Phaseoli vulgaris, ramis siccis Theobromae cacao (R 97), in humo aëreque, Honduras, Amer. centr. (O. A. Reinking no. R 97.)

Stroma pale, never carmine, conidia scattered, in pionnotes seldom in sporodochia, slender, attenuate at both ends, sickle-shaped, similar to *Fusarium herbarum,* pedicellate, 3-5-septate, 35-70 × 2.5–4.0 (30–82 × 2.5–4.5)μ; rarely 6-more septate, scattered conidia lanceolate, slightly curved, attenuate at both ends, apedicellate or appendicular; chlamydospores absent.

Habitat: In prematurely dead and rotting inflorescences of *Succisa pratensis.* In Berchtesgaden, Bavaria, Germany. (A. Braun.) On dead inflorescence of Rangphur lime (*Citrus aurantifolia*), on dead leaves of carrot (*Daucus carota*), on decaying peduncle and plant trash of banana (*Musa sapientum*), on dried pod of bush bean (*Phaseolus vulgaris*), on dead cacao twigs (*Theobroma cacao*) (R 97), and in soil and air. Honduras, Central America. (O. A. Reinking no. R 97.)

Fusarium anthophilum (A. Br.) Wr. is common on decaying and dead parts of various hosts. It can also be quite generally isolated from soil and air.

VIII. *Liseola Wr., Sherb., Rkg., Joh., et Bail.*

(Syn.. sect. Moniliforme Sherb.; subsect. Constrictum Wr.; pro parte sectionis Elegantis Wr.) Wollenweber, H. W., Sherbakoff, C. D., and Reinking, O. A., with the cooperation of Johann, Helen and Bailey, Alice A. 1924. Fundamentals for taxonomic studies of Fusarium. In Jour. Agr. Research 30: No. 2. 1925.

Microconidiis plus minusve in catenulis dispositis fusiodeo-ovoideis; macroconidiis subulatis, apice constrictis, basi pedicellata, forma et colore sectionis Lateritii, liberis, in sporodochiis, in pionnote, ochroleucis vel aurantiaco-cinnabarinis; chlamydosporis nullis; stromate expanso, ex albo

violaceo vel sclerotiis coeruleis erumpente. Status conidicus Gibberellae sectionis Liseae (Sacc.) Wr.

Microconidia more or less formed in chains, spindle to ovoid in shape; macroconidia slender with a slightly constricted top end, and a pedicellate base. Form and color similar to section Lateritium, scattered, in sporodochia or in pionnotes, brownish-white to orange-cinnamon; chlamydospores absent; stroma white to violet, spread out or erumpent, sometimes with blue sclerotia. Conidial stage of Gibberella section Liseae (Sacc.) Wr.

9. *Fusarium moniliforme* Sheld.

Sheldon, J. L. 1904. A corn mold (*Fusarium moniliforme,* n. sp.). Nebr. Agr. Exp. Sta., Ann Rpt., 1903, 17: 23–32, illus.

Saccardo, P. A. 1913. Sylloge Fungorum 22: *p. 1485.*

Wollenweber, H. W. 1917. Fusaria autographice delineata. Ann. Mycol. 15: *p. 23, fig. 366.*

Microconidiis catenulatim vel in capitulis falsis dispositis aerio mycelio albido-isabellino instratis, fusoideo-ovoideis, 5–12 × 2.25–4.0 μ; macroconidiis tenuibus subulatis, falcatis, attenuatis, pedicellatis, liberis, in sporodochiis, in pionnote, ochroleucis vel aurantiaco-cinnabarinis, plerumque 3-septatis, 30–36 × 3.0–3.5 (23–48 × 2.25–4.0) μ, rarius 1-, 4-, 5-septatis; 1-septatis, 12–18 × 2.25–3.5 μ; 4-septatis, 37–53 × 3.0–3.5 μ; 5-septatis, 43–66 × 3.0–3.5 μ; chlamydosporis nullis; sclerotiis coeruleis usque ad 0.5 mm. diam., stromate violaceo vel ochraceo.

Habitat: In caryopsidibus Zeae maydis aegrotis in Amer. bor. (Sheldon), in Honduras, Amer. centr. (O. A. Reinking, no. R 73), in foliis, intra truncum falsum maturum, in ramos floriferos emortuos Musae sapientum (R 53), in plantis indeterminates putridis, in humo aëreque, Honduras, Amer. centr. (O. A. Reinking no. R 53 et R 73), in foliis putrescentibus Ananassae sativae in insula Jamaica (C. G. Hansford no. 5, R 225).

Microconidia in chains or in false heads, formed in white to Isabella color aerial mycelium, spindle to ovoid in shape, 5–12 × 2.25–4.0 μ; macroconidia delicate and slender, sickle-shaped, attenuate, pedicellate, scattered or in sporodochia or pionnotes, brownish-white to orange-cinnamon; mostly 3-septate, 30–36 × 3.0–3.5 (23–48 × 2.25–4.0)μ, fewer 1-, 4-, 5-septate; 1-septate, 12–18 × 2.25–3.5 μ; 4-septate, 37–53 × 3.0–3.5 μ; 5-septate, 43–66 × 3.0–3.5 μ; chlamydospores absent; sclerotia blue, 0.5 mm. diam.; stroma violet or ochraceous.

Habitat: On diseased kernels of corn (*Zea mays*) in North America (Sheldon) and in Honduras, Central America (O. A. Reinking no. R 73), on leaves, in interior of diseased pseudostem and on dead floral parts (R 53) of banana (*Musa sapientum*), on decaying undetermined plant, and in soil and air. Honduras, Central America. (O. A. Reinking no. R 53 and R 73.)

On rotting bud leaves of pineapple (*Ananas sativus*). Jamaica. (C. G. Hansford no. 5, R 225.)

10. *Fusarium moniliforme* Sheld. var **erumpens** n. var.

A typo differt pluribus sclerotiis maioribus rugulosis atrocoeruleis modo Gibberellae acervatim erumpentibus; microconidiis catenulatim dispositis; macroconidiis plerumque 3-5-septatis; 3-septatis, 22–48 × 2.5–3.5 μ; 4-septatis, 33–47 × 3.25–3.5 μ; 5-septatis, 33–51 × 3.25–3.5 μ; chlamydosporis nullis.

Habitat: In disco trunci falsi vivnetis caesi Musae sapientum, Honduras, Amer. centr. (O. A. Reinking no. R 62.)

Differs from the type by having more and larger rugose, dark blue sclerotia, erumpent and clustered Gibberella like; microconidia in chains; macroconidia mostly 3-5-septate; 3-septate, 22–48 × 2.5–3.5 μ; 4-septate, 33–47 × 3.25–3.5 μ; 5-septate, 33–51 × 3.25–3.5 μ; chlamydospores absent.

Habitat: In vascular bundles of living, diseased pseudostem of banana (*Musa sapientum*). Honduras, Central America. (O. A. Reinking no. R 62.)

11. *Fusarium moniliforme* Sheld. var. **subglutinans** n. var.

A typo differt praesertim microconidiis non in catenulis dispositis; microconidiis continuis, 6–15 × 2.0–3.5 μ, macroconidiis plerumque 3-septatis, 21–38 × 3.0–3.5 (18–50 × 2.75–4.0) μ; rarius 1-septatis, 10–25 × 2.5–3.5 μ; 4-septatis, 27–50 × 3.25–4.0 μ; interdum 5-septatis, 43–55 × 3.25–4.0 μ; 6-7-septatis, 48–57 × 3.25–4.0 μ; chlamydosporis nullis; sclerotiis atrocoeruleis.

Habitat: In foliis et trunco falso putridis, in vascularibus fasciculis et ad superficiem trunci falsi viventis Musae sapientum, in aëre, Honduras, Amer. centr. (O. A. Reinking no. R 60.)

Differs from the type principally in having the microconidia not borne in chains; microconidia unicellular, 6–15 × 2.0–3.5 μ; macroconidia mostly 3-septate, 21–38 × 3.0–3.5 (18–50 × 2.75–4.0) μ; fewer 1-septate, 10–25 × 2.5–3.5 μ; 4-septate, 27–50 × 3.25–4.0 μ; sometimes 5-septate, 43–55 × 3.25–4.0 μ; 6-7-septate, 48–57 × 3.25–4.0 μ; chlamydospores absent; sclerotia dark blue.

Habitat: On decaying leaves and pseudostem, in the vascular bundles and exterior of living pseudostem of banana (*Musa sapientum*), and in the air. Honduras, Central America. (O. A. Reinking no. R 60.)

12. *Fusarium moniliforme* Sheld. var. **maius** n. var.

A typo differt macroconidiis longioribus, 3-5-(6)-septatis; 3-septatis, 28–48 × 2.25–3.5 μ; 4-septatis, 44–64 × 2.25–3.25 μ; 5-septatis, 54–76 × 2.5–

3.25 μ; 6-septatis, 61–92 × 2.5–3.25 μ: microconidiis catenulatim dispositis continuis, 4–16 × 2.0–4.0 μ, rarius 1-septatis, 14–21 × 2.5–3.5 μ; chlamydosporis nullis; sclerotiis atrocoeruleis.

Habitat: Ad pedunculos fructus, folia emortua Musae sapientum, Honduras, Amer. centr. (O. A. Reinking no. R 57.)

Differs from the type by longer macronconidia, 3-5-(6)-septate; 3-septate, 28–48 × 2.25–3.5 μ; 4-septate, 44–64 × 2.25–3.25 μ; 5-septate, 54–76 × 2.5–3.25 μ; 6-septate, 61–92 × 2.5–3.25 μ; microconidia in chains, unicellular, 4–16 × 2.0–4.0 μ; rarely 1-septate, 14–21 × 2.5–3.5 μ; chlamydospores absent; sclerotia dark blue.

Habitat: On dead peduncles and leaves of banana (*Musa sapientum*). Honduras, Central America. (O. A. Reinking no. R 57.)

13. **Fusarium neoceras** n. sp.

Microconidiis liberis vel in capitulis falsis, non in catenulis dispositis, continuis ovoideo fusoideis, 9–12 × 3.0–3.5 (5–18 × 2.75–4.5)μ; rarius 1-septatis, 19–26 × 3.5–4.5 (14–34 × 3.25–5.5)μ; macroconidiis in sporodochiis, plerumque vero in pionnote, elongatis subcurvatis, attenuatis, subpedicellatis, apice vix constrictis, 3–5-septatis, 38–68 × 4.0–5.0 (30–95 × 3.5–5.5)μ; 3-septatis, 32–59 × 3.0–5.5 μ; 4-septatis, 30–63 × 4.0–5.5 μ; 5-septatis, 55–67 × 4.5–5.5 μ, rarissimis 6–9-septatis, 67–120 × 4.0–5.0 μ; chlamydosporis et sclerotiis nullis; stromate interdum violaceo.

Habitat: In bracteis emortuis Musae sapientum et in humo, Honduras, Amer. centr. (O. A. Reinking no R 149.)

Microconidia scattered or in false heads, not in chains, unicellular ovoid fusoid, 9–12 × 3.0–3.5 (5–18 × 2.75–4.5)μ; rarely 1-septate, 19–26 × 3.5–4.5 (14–34 × 3.25–5.5)μ; macroconidia in sporodochia, but mostly in pionnotes, elongate, slightly curved, attenuate, subpedicellate, slightly constricted at the top, 3-5-septate, 38–68 × 4.0–5.0 (30–95 × 3.5–5.5)μ; 3-septate, 32–59 × 3.0–5.5 μ; 4-septate, 30–63 × 4.0–5.5 μ; 5-septate, 55–67 × 4.5–5.5 μ; very seldom 6-9-septate, 67–120 × 4.0–5.0 μ; chlamydospores and sclerotia absent; stroma sometimes violet.

Habitat: On dead floral bracts of banana (*Musa sapientum*) (R 149) and in soil. Honduras, Central America. (O. A. Reinking no. R 149.)

The various strains of Liseola herein described are widespread throughout banana plantations, being present on decaying plant trash of different kinds and on living parts of plants. The parasitic nature of the different strains has not been carefully tested.

IX. *Sectio Lateritium* Wr.

Wollenweber, H. W. **1917.** **Fusaria autographice delineata,** Ann. Mycol. 15: *p. 54.*

F. fructigenum Fr.

Fries, E. 1829. Syst. Myc. 3: *p. 471.*

Saccardo, P. A. 1886. Sylloge Fungorum 4: *p. 717.*

Wollenweber, H. W. 1917. Fusaria autographice delineata. Ann. Mycol. 15: *p. 19, fig. 281–285.*

Wollenweber, H. W. 1918. Conspectus analyticus Fusariorum. Berichte der Deut. Bot. Gesell. 35: *p. 740.*

14. *Fusarium fructigenum* Fr. var. *maius* Wr. forma 1 n. f.

Conidiis in sporodochiis et in pionnote, aurantiacis, fusoideo-falcatis, apicem versus conspicue inaequilatere et magis curvatis quam ad medium, utrinque constrictis atque adeo pedicellatis ad basim, 5(3–6)-septatis; 5-septatis, 48–64 × 3.5–4.5 μ; 6-septatis, 63–80 × 3.5–4.75 μ; 3-septatis, 34–44 × 3.5–4.5 μ; chlamydosporiis raris, sclerotiis (½ mm. diam.) atrocoeruleis vel ochroleucis; stromate carmineo. A typo differt colore carmineo stromatis.

Habitat: In planta ignota emortua, in insula Jamaica. (C. G. Hansford no. 16, R 235.)

Conidia in sporodochia and pinnotes, orange, spindle to sickle-shaped, dorsi-ventral difference in curvature more conspicuous towards the top cell than in the middle, constricted at both ends or even pedicellate at the base, 5(3–6)-septate; 5-septate, 48–64 × 3.5–4.5 μ; 6-septate, 63–80 × 3.5–4.75 μ; 3-septate, 34–44 × 3.5–4.5 μ; chlamydospores seldom present; sclerotia (½ mm. diam.) dark blue or ochraceous white; stroma carmine. Differs from the type by carmine color of the stroma.

Habitat: On undetermined dead plant. Jamaica. (C. G. Hansford no. 16, R. 235.)

The culture was obtained through the courtesy of Mr. C. G. Hansford of Jamaica.

XII. *Sectio Saubinetii* Wr.

Wollenweber, H. W. 1917. Fusaria autographice delineata. Ann. Mycol. 15: *p. 2.*

Stromate expanso flocoso vel plectenchymico, flavo-ochraceo, carmineo, mycelio aerio ex albo roseo intertexto; conidiis liberis, in sporodochiis, in pionnote, e pallide aurantiaco ochraceis, falcatis, elongatis, 3-pluriseptatis, apice rostrato, basi pedicellata vel—statu subnormi—apedicellata; chlamydosporis nullis. Aliquot species generi Gibberellae, sect. Saubinetii tribuendae.

Stroma spread out, floccose or dense, ochraceous, carmine, aerial mycelium from white to rose color; conidia scattered, in sporodochia or

in pionnotes, from pale orange to ochraceous, sickle-shaped, elongate, 3-more septate, constricted at top end, pedicellate at base, sometimes apedicellate; chlamydospores absent. Some species have a perfect stage that is a Gibberella of section Saubinetii.

15. **Fusarium macroceras** n. sp.

Stromate fioccoso ex albo roseo vel plectenchymico, flavo, ochracco. carmineo; conidiis liberis lanceolatis vel subfalcatis, dorsiventralibus, ad apicem rostratis, ad basim conicis, apedicellatis; conidiis in sporodochis et in pionnote elongatis, subfalcatis, utrinque attenuatis pedicellatis, 5-7-septatis, 47–64 × 4.5–5.75 (35–74 × 4.0–7.0)µ, rarius 1-4-vel 8-9-septatis, rarissime 14-septatis, 150 × 625 µ; chlamydosporis nullis.

Habitat: In leguminibus maturis Phaseoli vulgaris, Honduras, Amer. centr. (O. A. Reinking no. R 95.)

Stroma floccose, rosy white, or dense plectenchymic, yellow, ochraceous, and carmine. Conidia scattered, lanceolate or sickle-shaped, dorsi-ventral, constricted at the top end, conical at the base, apedicellate; conidia in sporodochia and pionnotes, elongate, slightly sickle-shaped, attenuated at both ends, pedicellate, 5-7-septate, 47–64 × 4.5–5.75 (35–79 ×3.5–7.0)µ, fewer 1-4- or 8-9-septate, very seldom 14-septate, 130 × 6.25 µ; chlamydospores absent.

Habitat: On mature bush bean pods (*Phaseolus vulgaris*). Honduras, Central America. (O. A. Reinking no. R 95.)

XIII. *Sectio Elegans* Wr.

Wollenweber, H. W. 1913. Studies on the Fusarium problem. Phytopathology 3: *p. 28.*

Wollenweber, H. W. 1918. Conspectus analyticus Fusariorum. Berichte der Deut. Bot. Gesell. 35: *p. 741.*

Subsectio Orthocera Wr.

Wollenweber, H. W. 1917. Fusaria autographice delineata. Ann. Mycol. 15: *p. 2.*

Wollenweber, H. W. 1918. Conspectus analyticus Fusariorum. Berichte der Deut. Bot. Gessell. 35: *p. 741.*

16. **Fusarium bostrycoides** n. sp.

Stromate plectenchymico ex ochroleuco viridi vel violaceo; aërio mycelio caespitoso flavido albo; microconidiis numerosis, instratis vel in capitulis falsis ad conidiophoros verticillate et bostryce ramosos dispositis, continuis, ovoideis, 6–11 × 2.5–3.24 (4–13 × 2.0–4.0) µ, rarissimis 1-septate, 15–22 ×

2.5–3.75 μ, 3-septatis orthoceris vel leniter falcatis, subpedicellatis, 24–29 × 2.5–4.0 μ; sporodochiis et pionnote deficientibus; chlamydosporis numerosis terminalibus et intercalaribus, globosis, singulis, vel catenulatis, rugulosis, 6–8 μ diam.

Habitat: In humo, Honduras, Amer. centr. (O. A. Reinking no. R 169.)

Observatio: Fungus ad pultem Oryzae coctae cultus colorem roseum acidum secernit, cuius modificatio alcalina coerulea est.

Stroma plectenchymic from brownish white to green or violet; aerial mycelium caespitose, cream color; microconidia, numerous, scattered or in false heads, formed on verticillate or bostrix-like branched conidiophores; unicellular, ovoid, 6–11 × 2.5–3.25 (4–13 × 2.0–4.0) μ; very rarely 1-septate, 15–22 × 2.5–3.75 μ, and 3-septate, straight to slightly sickle-shaped, subpedicellate, 24–29 × 2.5–4.0 μ; sporodochia and pionnotes absent; chlamydospores numerous, terminal and intercalary, globose, unicellular or in chains, rugose, 6–8 μ diam.

Habitat: In soil, Honduras, Central America. (O. A. Reinking no. R 169.)

Note: Fungus on rice culture with rosy acid modification of color, changing to blue by addition of sufficient alkali.

XIV. *Sectio Martiella* Wr.

Wollenweber, H. W. 1913. Studies on the Fusarium problem. Phytopathology 3: *p. 30.*

Wollenweber, H. W. 1918. Conspectus analyticus Fusariorum. Berichte der Deut. Bot. Gesell. 35: *p. 738.*

17. **Fusarium alluviale** n. sp.

Stromate erumpente ruguloso badio vel aerugineo tincto; conidiis dorsiventralibus fusoideo-falcatis, apice truncate rostrato, basi subpedicellata, in sporodochiis, in pionnote vel liberis, in massis ex albo badiis, 3-septatis, 29–34 × 4.25–5.5 (25–44 × 4.0–6.25) μ; rarius 4-septatis, 32–43 × 4.0–6.0 μ; in aërio mycelio conidiis minoribus quoque, continuis, 9–14 × 3.0–4.5 μ, et 1-septatis, 15–25 × 4.0–5.25 μ; chlamydosporis terminalibus et intercalaribus, interdum spinosis, in mycelio et in conidiis, plerumque continuis, 6–11 μ diam. Fungus in variis substratis cultis gravem odorem reddit.

Habitat: In humo alluviali, Honduras, Amer. centr. (O. A. Reinking no. R 188.)

Stroma erumpent, rugose, greenish blue; conidia dorsiventral, spindle to sickle shaped, top cell beak shaped, basal cell slightly pedicellate, in sporodochia, in pionnotes or scattered, in masses from white to chestnut-brown; 3-septate, 29–34 × 4.25–5.5 (25–44 × 4.0–6.25) μ; seldom 4-septate, 32–43 × 4.0–6.0 μ; in aerial mycelium smaller conidia also present, unicellular, 9–14 × 3.0–4.5 μ, and 1-septate, 15–25 × 4.0–5.25 μ; chlamydospores terminal, intercalary, sometimes spiney, in mycelium and in conidia, mostly unicellular, 6–11 μ dia. Strong odor produced on various culture media.

Habitat: In alluvial soil. Honduras, Central America. (O. A. Reinking no. R 188.)

18. ***Fusarium theobromae*** App. et Strk.

(Syn. ***Fusarium heveae*** P. Henn.)

Appel, O, and Strunk, H. F. 1904. Uber einige in Kamerun auf Theobroma cacao beobachtete Pilze. Centralbl. f. Bakt. u Par. etc. Abt. II. 11: 551–557, 632–637.

Wollenweber, H. W. 1917. Fusaria autographice delineata. Ann. Mycol. 15: *p. 26, fig. 426–428.*

Stromate plectenchymico ochroleuco interdum olivaceo vel aeruginoso; aërio mycelio ex albo cremeo intricato, caespitoso; conidiis plerumque sparsis interdum in sporodochiis et in pionnote, cremeis, elongatis leniter incurvatis, 3–5-septatis; 3-septatis; 28–46 × 3.5–5.0 μ; 5-septatis, 44–60 × 4.0–5.5 μ; rarissime 6-septatis, 52–73 × 4.5–5.5 μ,[5] in aërio mycelio quoque minoribus plus minusve continuis, ovoideis, 6–12 × 2.5–3.5 μ; chlamydosporis globosis vel pyriformibus, terminalibus et intercalaribus, singulis, binis, acervalibus, 5–8 μ diam.

Habitat: In fructibus seminibusque putridis Theobromae cacao, in horto botanico Victoriae Camerun Africae occid. (Strunk), ramis emortuis Heveae brasiliensis, Ceylon, Indiae, radice Manihotis utilissimae, Java; et in humo, Honduras, Amer. centr. (O. A. Reinking no. R 129.)

Stroma plectenchymic, ochraceous white, sometimes olive to greenish blue; aerial mycelium cream white, caespitose; conidia mostly scattered, more seldom in sporodochia, cream color in masses, elongate, slightly curved, 3–5-septate; 3-septate, 28–46 × 3.5–5.0 μ; 5-septate, 44–60 × 4.0–5.5 μ; very rarely 6-septate, 52–73 × 4.5–5.5 μ;[6] in aerial mycelium also smaller conidia, mostly unicellular ovoid, 6–12 × 2.5–3.5 μ; chlamydospores globose or pear shaped, terminal and intercalary, one celled, two celled or in clusters, 5–8 μ diam.

Habitat: On rotted fruits and kernels of cacao (*Theobroma cacao*) in botanical gardens Victoria, Camerun, Western Africa (Strunk); dead branches of Hevea rubber (*Hevea brasiliensis*) Ceylon, India; roots of cassava (*Manihot utilissima*) Java, and in soil, Honduras, Central America. (O. A. Reinking no. R 129.)

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[5] Conidia in diagnosi sec. Appel et Strunk pluri-septata, 45–75 × 5.0–7.0 μ; conferas vero conidia in Wollenweber, Fus. del. 428, 4–6 (3–7)-septata, 39–60 × 4.5–5.25 μ.

[6] Conidia according to the diagnosis of Appel and Strunk are described as pluriseptate, 45–75 × 5–7 μ, while according to Wollenweber, in Fus. del. 428, they are 4–6 (3–7)-septate, 39–60 × 4.5–5.25 μ.

19. **Fusarium ensiforme** n. sp.

Stromate erumpente sclerotiali rugoso saepe atrocoeruleo; conidiis in sporodichiis et in pionnote ex albo aureo-ochraceis, elongatis subfalcatis, apice leniter constricto, basi conspicue pedicellata, 5–6 (3–7)-septatis, 55–72 × 4.5–5.0 µ; 7-septatis, 69–81 × 4.75–5.0 µ; 4-septatis, 43–60 × 4.0–5.0 µ; 3-septatis, 37–50 × 3.75–5.0 µ; in aërio mycelio quoque minoribus, plerumque continuis ovoideis vel subcurvatis, 5–12 × 2.5–4.0 µ; chlamydosporis terminalibus vel intercalaribus, singulis vel binis, interdum rugosis, 6–9 µ diam.

Habitat: Ad fructus putridos Fici spec. ignotae arboris procerae tropicalis, in silva nativa. Honduras Amer. centr. (O. A. Reinking no. R 88.)

Stroma erumpent, sclerotial rugose, often dark blue; conidia in sporodochia and in pionnotes, from whitish to golden yellow, elongate, slightly sickle shaped, somewhat constricted at the top end, distinctly pedicellate at the base, 5–6 (3–7)-septate, 55–72 × 4.5–5.0 µ; 7-septate, 69–81 × 4.75–5.0 µ; 4-septate, 43–60 × 4.0–5.0 µ; 3-septate, 37–50 × 3.75–5.0 µ; in aerial mycelium also smaller, mostly unicellular, ovoid or slightly curved, 5–12 × 2.5–4.0 µ; chlamydospores terminal or intercalary, one- or two-celled, sometimes rugose, 6–9 µ diam.

Habitat: On decaying fruit of wild fig (*Ficus* sp.) in virgin forest. Honduras, Central America. (O. A. Reinking no. R 88.)

CONCLUSION

The general type of macroconidia, which was regarded as the most important character in former taxonomy of Fusaria, has now been proved to be more valuable in connection with other characters, such as the presence of microconidia, chlamydospores, and sporodochial or pionnotal production. The newly established section of Liseola has certain characters which distinguish it from other related sections. It could only be separated from section Lateritium by having microconidia which in general are absent in the latter. Section Elegans has chlamydospores that are not produced in section Liseola. The separation from section Roseum is based partially on a tendency to a constriction of the top end-cell of the macroconidia, which is rather characteristic in section Liseola and also by the presence of typical Liseola microconidia. The relationship of Lateritium and Liseola, on the other hand, is proved by the presence of a Gibberella with similar ascospores as a perfect stage in both groups. Section Saubinetti has a Gibberella as a perfect stage, but with more sickle-shaped ascospores.

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NEW STUDIES ON STIPPLE-STREAK DISEASE OF POTATO

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With Two Figures in the Text

Various workers have shown during the last six or seven years that the so-called "virus" or mosaic disease of plants may overwinter in certain perennials in some instances and that some of the mosaic diseases can be brought over to plants on which they produce no pathologic changes, but in which their virus increases and from which plants it can be recovered with great ease and in very virulent form.

All this, however, does not appear to account for the sudden appearance and spread of some of these diseases under all conditions. Many and different may prove to be the explanations of such cases when the nature of these diseases becomes better understood.

The writer's studies on the stipple-streak disease of potato during the last few years, the results of which are discussed below, throw some new light on the mosaic problem. They appear to explain, partially at least, some of the many difficulties which the student of potato mosaic diseases has so often to deal with.

One of the common difficulties is the appearance of an entirely different disease in the artificially infected plants, even when all possible precautions have been taken to avoid external infection by insects or contamination of the virus. This apparently indicates that some and perhaps all of these diseases may be present in some potato plants or varieties in a "latent" form.

In Professor Quanjer's long experience with these diseases, as well as during the few years of the writer's work on them, stipple-streak was the disease which appeared most commonly on plants and in plots where it has been least expected.

The stipple-streak disease of potato, as already pointed out in the writer's first paper (1) on this subject, resembles superficially, in its primary form at least, some of the bacterial and fungus diseases of plants. It is, however, a "virus" disease *par excellence* and differs principally in no way from the infectious mosaic diseases of potato. Roughly speaking, it is a magnification of a mosaic disease. Every characteristic of the mosaic diseases—symptoms, infection period, effect on infected plants, and yield—is to be seen in it in a highly intensified form, which naturally leads to the appearance in some cases of new symptoms and conditions alien for the mosaic diseases. Working with this disease is therefore easier. This disease is unquestionably the most suitable object for fundamental studies on the degeneration diseases of potato.

It is generally known that not all potato varieties are equally susceptible to the different virus diseases of this plant. Some of them show more pronounced symptoms of such diseases and their yields deteriorate much more rapidly under their influence, while other varieties do this less. Still other varieties have never been seen to suffer from some of these diseases, though careful examination of such varieties has often shown that they are as subject to the disease or diseases in question as any other variety.

The various potato varieties are attacked in different degrees also by the stipple-streak disease, but whether this means that some varieties are more, others less, susceptible to this disease is not certain. It is certain, however, that plants of different varieties when attacked by this disease show in very different degrees and forms the effects of the infection; some of them are severely affected, they wilt and die in a short time, like the variety Schotsche Muis. Others, like the variety Non Plus Ultra, are much less so. While still others, though infected, are in no way injured or affected and continue giving an almost normal yield, even under extremely favorable conditions for the development of the disease. Such are the varieties Ashleaf and Koksiaan.

All these varieties are therefore susceptible to stipple-streak, but they possess a different "sensitiveness" for it. Whether there are potato varieties actually resistant to stipple-streak, in the true sense of the word, is not known and not very probable.

The existence of varieties such as the last two mentioned, which represent a kind of masked carrier of one of the most destructive and troublesome virus diseases of potato, is of great practical and scientific importance: First, because it makes possible the breeding of resistant varieties, but primarily because such varieties represent a great danger for the more "sensitive" potato varieties, that are susceptible to this disease. Such varieties are unquestionably a very important permanent source of inoculum in nature.

During the spring of 1921 there appeared a heavy stipple-streak epidemic in the districts of early potato culture in Holland, primarily in the variety known in Holland under the name Schotsche Muis and in England under that of Midlothian Early. This variety, which is the most productive and widely spread early variety in Holland, is the most sensitive of varieties susceptible to this disease, so far as the writer knows. Most infected plants were dead before the beginning of June. Few of them had formed any tubers.

The very rapid development of the disease and the very early death of the infected plants of this variety should naturally lead to a better knowledge of this disease. But in spite of this the disease appears in a smaller or larger degree every year.

During the summer of 1921 the stipple-streak disease appeared in a lesser degree and in a lighter form also in various other varieties. The variety Non Plus Ultra, among other varieties, showed typical primary symptoms of stipple-streak, but the plants remained alive and green almost as long as the healthy plants of the same variety did and gave a normal yield. The varieties Ashleaf and Koksiaan,[1] which stood near plots of Schotsche Muis, where 75 per cent of the plants were infected, showed, upon a superficial examination, no symptoms of the disease. Both varieties were in excellent condition. A careful examination of their leaves, however, showed that some of them had one or more typical stipple-streak stipples or streaks, especially on their lower side. In these two varieties as well as in all varieties which are not very sensitive to the disease, the necrotic stipples and streaks appear first after the leaves have reached their full size so that as a rule they do not lead to the crinkling and distortion of the leaves, as is the case in the highly sensitive varieties.

Those plants of the above varieties which showed some streaks or stipples were considered as infected and their tubers were collected for further study.

During the following spring tubers of the above four varieties (Schotsche Muis, Non Plus Ultra, Ashleaf and Koksiaan) collected from stipple-streak plants were planted in special rows.

The Schotsche Muis tubers sent out few short and highly degenerated, curled, and crinkled plants, which died shortly after coming above the ground and formed none or very small tubers.

The Non Plus Ultra tubers gave rise to apparently normal and vigorous plants, on the leaves of which later appeared typical and numerous stipples and streaks. The plants remained alive almost as long and gave almost as good a yield as the healthy plants of the same variety did, though they were of a slightly smaller size than the healthy plants. All tubers showed brown necrotic areas and blisters, as is common of the Schotsche Muis stipple-streak tubers.

The tubers of Ashleaf and Koksiaan stipple-streak plants upon planting gave absolutely normal plants which remained in every respect as the healthy plants of the same variety and gave a good yield. Only about 10 per cent of the hills, which numbered eighty of each variety, had more or less black stipples and streaks on their otherwise normal leaves. The tubers of all plants of both varieties, however, upon digging showed distinct symptoms of the disease. The Ashleaf tubers showed brown necrotic areas and blisters deep under the skin of the tubers, resembling somewhat Phytophthora infections, while those of the variety Koksiaan had a uniform dirty gray or brown skin.

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[1] Known in England under the name Jersey Non Such.

The tubers of all of the above varieties harvested during the summer of 1922 were planted again in the spring of 1923 and gave similar results. The Schotsche Muis plants formed no tubers in the second generation of the disease, so that for the years 1923 and 1924 new tubers of primary stipple-streak plants were used. The progenies of the plants grown during the

FIG. 1. Stipple streak infected potato plants of different varieties, of same age and grown under same conditions. Photographed June 15, 1924.

a Two Schotsche Muis plants from originally healthy tubers, which tubers have been infected with stipple streak, two and a half months before planting and four months before this photo was taken, by allowing infectious aphids to suck on their sprouts for 10 days The plants are very dwarf and degenerated with curled and crinkled leaves, lower leaves dropping off and rusted, no new tubers are formed.

b. One Stem plant, progeny of a primary stipple streak plant, infected during the summer of 1923. Leaves curled and crinkled with numerous brown streaks and stipples. Lower leaves dropping off. Some new tubers are formed.

c. One Non Plus Ultra plant, fourth year stipple-streak infected, quite normal, but smaller than the healthy plants, leaves with deeper venation and with numerous stipples and streaks. Few quite large tubers are formed

d and e. Ashleaf and Koksiaan plants, respectively; both fourth year stipple-streak infected. No symptoms of the disease are to be seen on the otherwise vigorous, normal, and healthy plants, except on the newly formed large and numerous tubers.

summer of 1923 were planted for the third time during the spring of 1924 with practically the same results as at the beginning of the experiment in 1922. The variety Schotsche Muis already during the second year was so degenerated that no new tubers were formed. The variety Non Plus Ultra gave during the second, third and fourth generation somewhat smaller plants than the healthy ones, but during all of the three vegetative periods formed quite large tubers, though the yield in general was noticeably decreased. Ashleaf and Koksiaan showed no degeneration or running out in any way, except perhaps a slight decrease in yield.

During the winter of 1923–24 ten tubers of the infected material of each of the above four varieties were grafted on healthy tubers of the variety Schotsche Muis. In all cases the infection passed over into the grafted healthy tubers, which developed typical symptoms of the disease as is known for this variety. This shows that the material of the varieties Non Plus Ultra, Ashleaf, and Koksiaan used in the above field experiments actually did carry the virus of the stipple-streak disease.

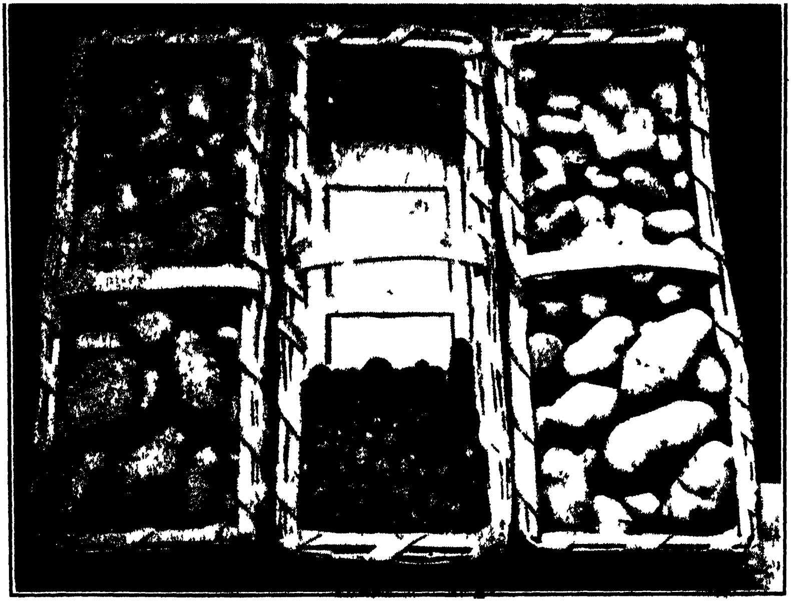

FIG. 2. On the left and right are shown the yields of ten hills of Ashleaf and Koksiaan, respectively; both fourth year stipple-streak sick and belonging to the plants pictured in figure 1. In the middle is shown the yield of 60 (sixty) Schotsche Muis plants from originally healthy tubers, which tubers were artificially infected with stipple streak by allowing infectious aphids to suck on their sprouts for 10 days, then incubated at 18° C. for two months and planted. These are the tubers of plants like those pictured in figure 1. All three varieties were harvested at the same time and when fully ripe.

During the summer of 1924 five young healthy Schotsche Muis plants were grafted with tops of each of the varieties Ashleaf and Koksiaan to see whether the plants were still infectious. The Ashleaf and Koksiaan plants from which was taken material for grafting, though originating from the slightly stipple-streak plants observed during the summer of 1921, showed absolutely no symptoms of the disease and were in every respect very normal, vigorous, and free from any disease, with exception of their tubers, which showed pronounced symptoms of the stipple-streak disease.

All of the grafted plants became infected and developed typical and very heavy symptoms of stipple-streak within 2–3 weeks, both on the leaves and tubers. All check plants remained healthy. All precautions were taken to protect the plants from coming in contact with insects.

This experiment proves definitely that the varieties Ashleaf and Koksiaan which, when infected with stipple-streak, may show slight primary symptoms of this disease only in very rare cases, while in the great majority of cases they show no symptoms whatever on their aerial portions, even in the third generation after the infection.

Under such conditions the probability that such varieties may eventually become wholly infected in all their lines and selections, provided they are visited by the stipple-streak transmitting aphids, is very great. It can therefore be taken for granted that at least a portion of the Ashleaf and Koksiaan plants under field conditions are infected with stipple-streak and carry in abundance the "virus," thus forming dangerous masked sources of infection for the more sensitive potato varieties.

In the old literature on the degeneration and running out of potato varieties has been described repeatedly a condition which resembles very much the condition described above. Simon, in 1782, for instance, writes that the first cases of degeneration in potatoes in Germany were noticed at the time (1770) when the so-called Viehkartoffel was introduced.[2] Before the introduction of this potato variety from England, he states, the disease had never been seen. The more common the growing of this variety became, the more common became also the disease among all other varieties, with the difference only that in some varieties the disease appeared more and spread more rapidly, in others less so. Localities where this variety was not grown remained free from the curl disease for a long time after, until at last, with the general introduction of this variety, the whole country became infected. The Viehkartoffel itself, writes Stockmar, as has been noticed also by Simon, Buchan-Hepburn, Ackermann, etc., (2) "does not suffer from the curl disease and even though much poorer in quality, originally used only as animal food, it gradually replaced in most localities the common varieties, both red and yellow, which did suffer much from the disease.''

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[2] This variety was introduced in Germany from England in 1770 at the time of the great food scarcity. It is a very prolific—but unsuited for human consumption—and unusually late variety, which has never hitherto been injured by the curl. It is stated that it had been brought by a sailor from America and has been known in England ever since 1711 under the name Yam or Surinam potato.

The cause of the disease was considered by the early writer on this subject to lie in the cross pollination of the common potato varieties by the Viehkartoffel.

Stockmar (2), in 1801, made some experiments to establish the correctness of the above hypothesis. For this purpose he took tubers from the susceptible potato varieties from localities where the disease was not known. One portion of these tubers he planted away from, and the other portions next to, Viehkartoffeln. The first year all plants remained healthy. The second year he planted the tubers of the potatoes that had been next to Viehkartoffeln away from such potatoes and planted again other healthy potatoes near the Viehkartoffeln. Of the potatoes which the first year had been next to the Viehkartoffeln, he writes: ''After the first leaves had hardly appeared above the ground I saw what I had suspected; more than half of these plants were curled, while the potatoes grown the previous year away from Viehkartoffeln, also those which this year are next to the latter did not show even the slightest symptoms of curling.''

Unfounded as the old hypotheses regarding the nature of the curl disease may be, Stockmar's experiments prove nevertheless that the Viehkartoffel must have been a masked carrier of some mosaic disease, while the fact that in the second generation the plants showed distinct curling as soon as the first leaves appeared above ground justifies the supposition that the disease in question must have been stipple-streak, since it is the only one of the degeneration diseases by which the infected plants in the second generation show severe curling of the leaves as soon as they appear above the ground (1) provided that they are of a sensitive potato variety.

What has been said above of the varieties Ashleaf and Koksiaan may prove to be true also of other varieties, or of individual plants of other varieties, including even some of the most sensitive varieties to this disease. This supposition is somewhat strengthened by the recent work of Dorst (3) who has shown that a potato variety is in no case a genetically pure line, on account of the common occurrence of bud mutations.

Similar conditions as described above for stipple-streak must naturally exist in connection with other virus diseases of this plant, though so far this has not yet been experimentally established.

Oortwijn Botjes (5) and Murphy (4) have observed conditions which may be considered as similar with the condition treated in this paper, but they are of an entirely different nature. Oortwijn Botjes writes that in one case, probably under the influence of the weather conditions, the progeny of a mosaic plant failed to show any symptoms of the disease, but apparently served as a source of infection for the neighboring plants. Murphy observed that under certain climatic conditions the symptoms of potato mosaic become less apparent or may completely disappear. The plants apparently recover from the disease and give a quite normal yield. When these plants or their progenies are grown again under conditions favorable for the development of the disease, they show its presence again.

SUMMARY

The potato varieties susceptible to stipple-streak possess a widely differing "sensitiveness" to this disease.

Some varieties, though infected by the disease, show no or only very slight symptoms of same, under all conditions. In other words, such varieties are masked carriers of the virus of this disease under field conditions.

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SCAB OF CHRISTMAS BERRY, PHOTINIA ARBUTIFOLIA LINDL., DUE TO FUSICLADIUM PHOTINICOLA N. SP.

R. L. McClain

With Two Figures in the Text

About the year 1912 Professor W. T. Horne of this Division became interested in a scab disease occurring on the California Christmas berry, *Photinia arbutifolia* (*Heteromeles arbutifolia* Roerm.) This disease was found on leaves and berries and closely resembles scab on pears and apples. At his suggestion the writer has made a study of the fungus causing Christmas berry scab, particularly in comparison with the pear scab fungus (*Venturia pirina* Aderh.), which it seemed most closely to resemble.

The Christmas berry is a plant peculiar to California and the islands off the coast. It is an evergreen shrub with many branches given off from a short, thick trunk. The leaves are tough, leathery, shiny and of deep green color. The berries, which are bright red, are borne in large clusters from October to February. The branches and berries make a striking appearance and are much used for decorations.

DESCRIPTION OF THE DISEASE

As already stated, the symptoms of the scab on the Christmas berry are very similar to those on the apple and pear. The fungi are confined to the surface layers of cells of the hosts but the tissue beneath may be killed. If the Christmas berry leaves are attacked when quite young, they may be killed, while infection occurring after the leaves have hardened (Fig. 1) produces more or less rounded, olive-green spots on either side. Old infections become gray in the center, surrounded by an olive-green, slightly zonate margin of new conidia. These old leaves may change from green to various shades of red and brown. On the green berries the scabby patches almost completely cover the surface, causing them to shrivel. In the winter of 1923–24 the scab was not at all common around Berkeley, California, and in the fall of 1924 no infection was found. From these observations we are led to believe that the fungus does very little damage. In the wild state the Christmas berry is host to so many insect pests that it is difficult to determine the injury from any one organism.

METHODS

The Christmas berry scab fungus was isolated in single-spore cultures by both hanging-drop and plate methods. The spores were taken from typical scab spots on the leaves of the Christmas berry collected from a shrub in the Northbrae district of Berkeley, California, in September, 1923. The pear scab fungus was isolated by the same methods from a pear fruit in October, 1923.

In order to make comparative studies of the fungi, the following culture media were used: standard nutrient agar, prune juice, prune agar, bread and prune juice, bread and Christmas berry leaf juice, cornmeal and pear juice, and steamed rice.

Fifteen cultures of each fungus in each of the above series, twelve for inoculation and three for checks, were used throughout this study. Single-spore cultures secured by the plate method were used in making transfers. All cultures were kept in the laboratory at room light and temperature.

Inoculations were made in the laboratory and in the greenhouse both on Christmas berry and pear trees, and in the field on Christmas berry. The spores or mycelium were either laid on the wet leaves or the spores sprayed on with an atomizer. Both pure cultures of the fungi and spores produced on the respective hosts were used.

COMPARATIVE MORPHOLOGY

Only the imperfect or Fusicladium forms of the fungi were found and studied. The chief differences are found in the shape and character of the conidiophores and conidia (Fig. 2). The conidiophores of both fungi are about 8 × 30 μ in size, usually one-celled and olive-green in color. Those occurring on the Christmas berry are smooth, usually slightly enlarged at the end and at the base, while those of the pear scab fungus are distinctly knobby, suggesting that several spores had been given off from a sporophore. The conidia of both fungi vary greatly in size and shape, particularly those of the pear scab fungus. The conidia of the Christmas berry scab fungus are about 10 × 22 μ average size, distinctly pyriform, broadly truncate at base, outer end well rounded, the greatest diameter below the middle. In contrast, those of the pear scab fungus are about 8 × 22 μ average size, very slightly pyriform, narrowly truncate or not at all, frequently well pointed at both ends, widest part typically near the middle.

It will be noted by the chart that the pear scab fungus in cultures is uniformly darker in color than the Christmas berry scab fungus. The most striking difference is found on steamed rice in which the former is almost black while the latter is the characteristic olive-green. These differences were so pronounced that the fungi could easily be distinguished from each other.

In general, conidia were produced in abundance by the pear scab fungus while none or very few were produced by the Christmas berry scab fungus.

TABLE 1.—*Comparative cultural characteristics of Fusicladium pirina and F. photinicola*

Medium	Fungus	Color*	Cultural Characteristics
Nut. agar	Chr. b. scab	Deep grayish olive	Tough button of mycelium in center of colony, mycelium regular—*i. e.*, not breaking up into chlamydospores, very few conidia.
	Pear scab	Dark olive	Almost all of mycelium breaking up into chlamydospores, conidia abundant.
Bread and prune juice	Chr. b. scab	Deep olive	Tough mycelium, regular, no conidia.
	Pear scab	Dark olive	Chlamydospores abundant, conidia numerous.
Steamed rice	Chr. b. scab	Deep grayish olive	Mycelium regular, no conidia.
	Pear scab	Deep olive	Mycelium regular, conidia fairly numerous.
Corn meal and pear juice	Chr. b. scab	Deep olive	Mycelium very compact, tough, an occasional conidium.
	Pear scab	Dark olive	Mycelium very smooth, tough, an occasional conidium.
Bread and Chr. b. leaf juice	Chr. b. scab	Olive green	Mycelium regular, very few conidia.
	Pear scab	Olive green	Mycelium having numerous enlargements, conidia abundant.

* Ridgway's Color Standards.

From these and the following we are led to believe that the fungus causing scab on the Christmas berry is distinct from that causing scab on the pear.

INOCULATIONS

While no infection was secured in the field or in the greenhouse by either fungus, infection of the Christmas berry scab fungus on the leaves of Christmas berry plants growing under bell jars in the laboratory was secured by using spores produced in a natural scab infection. No infection was secured on pear leaves under the same conditions.

IDENTIFICATION

Other Fusicladia known to occur in California are: apple scab, *Venturia pomi* Aderh.; loquat scab, *Fusicladium eryobotryae* Sciala.; and an unidentified species attacking *Cotoneaster crenulata.* While no cultural studies of these fungi have been made, it appears from their descriptions that they

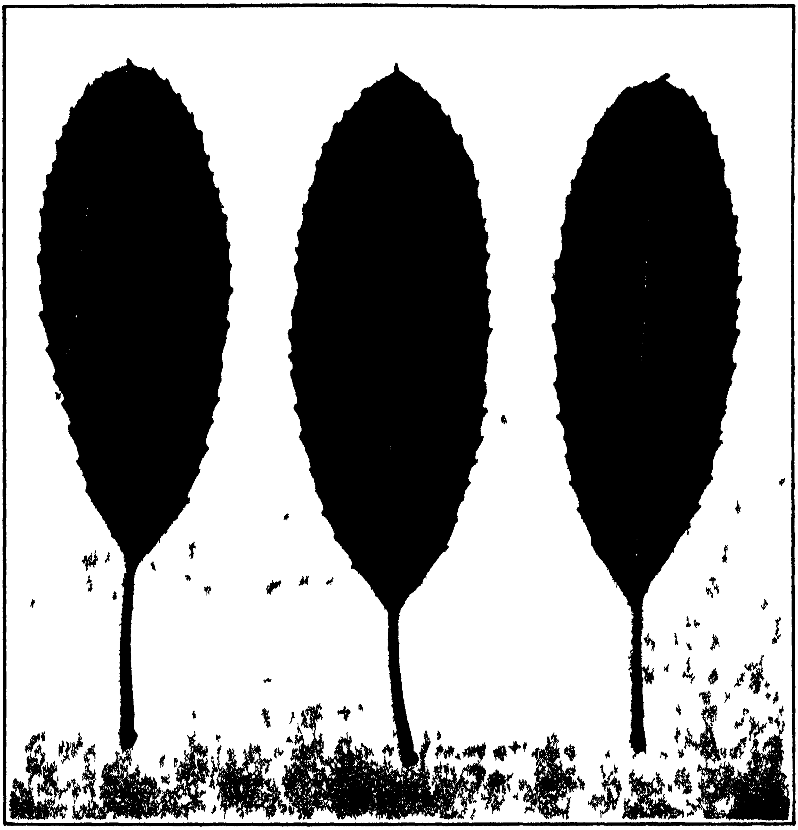

Fig. 1. Scab infection on lower side of Christmas berry leaves.

are distinct from the fungus causing Christmas berry scab. For these and the foregoing reasons it seems advisable to describe the latter as a new species.

Fusicladium photinicola n. sp.

Conidiophores 8 × 30 µ in size, usually one-celled, olive-green in color, smooth, slightly enlarged at both ends. Conidia average about 10 × 22 µ in size, distinctly pyriform, broadly truncate at base, other end well rounded, largest diameter below the middle, olive-green in color. Mycelium of same color, 5–7 µ in diameter; in cultures, the colonies under the low power of the microscope showing radiating, irregular, wavy threads of

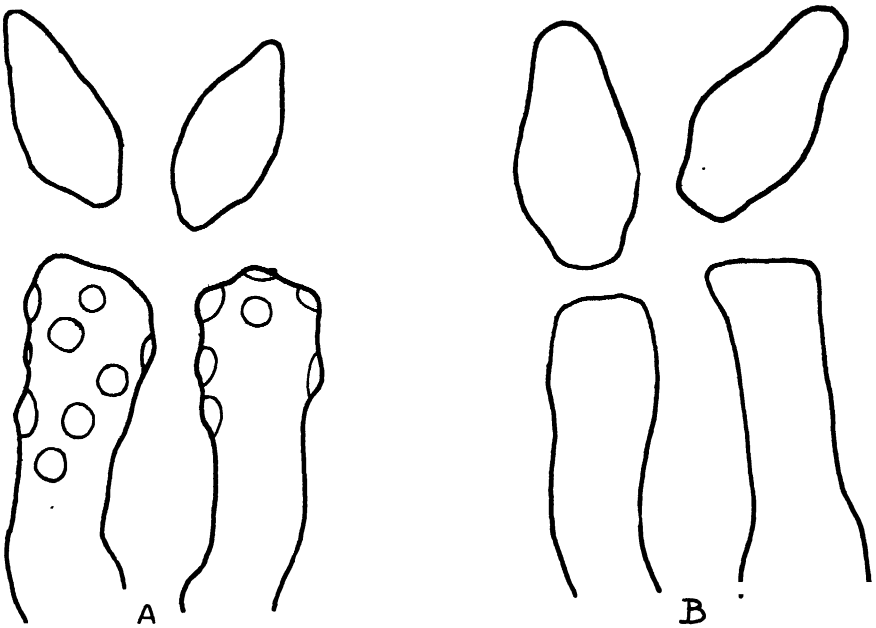

FIG. 2. Conidia and conidiophores, × 500. (A) *Fusicladium pirina,* (B) *F. photinicola.*

mycelium and producing appresoria at the ends of certain filaments. The conidia germinate readily, but the fungus grows very slowly in cultures.

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THE EFFECT OF CALCIUM CARBONATE ON BORDEAUX MIXTURE

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The use of hydrated lime in the preparation of Bordeaux has brought up the question of calcium carbonate as an impurity of the lime, and its action on the physical properties of the mixture. Data on this question

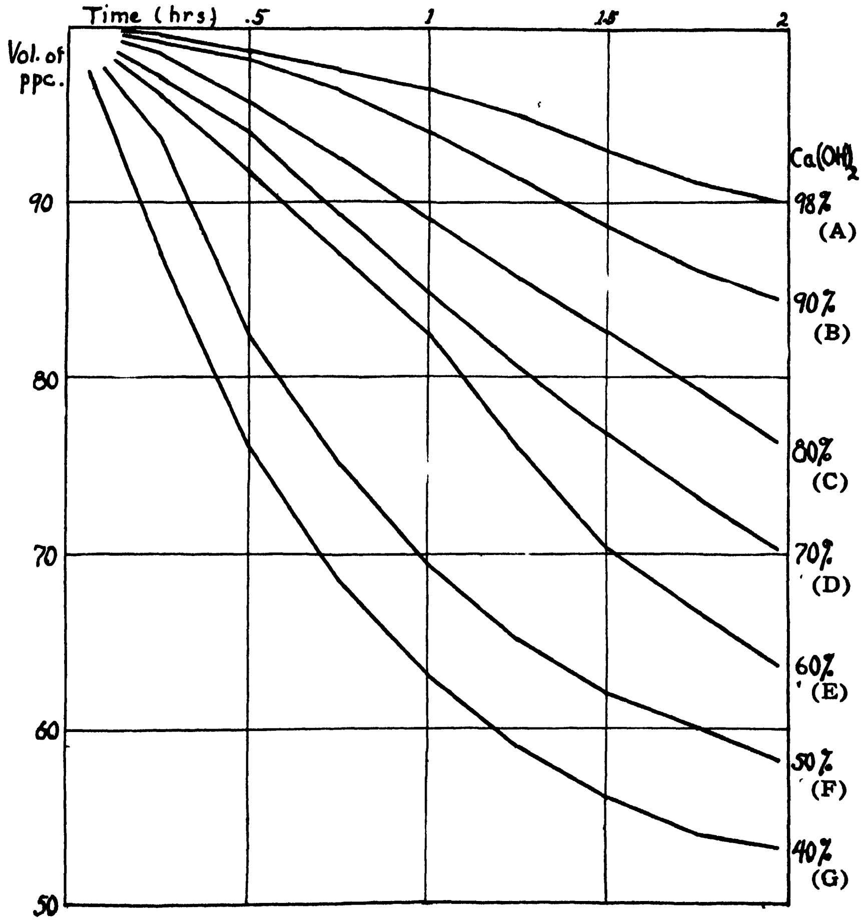

Fig. 1

Average rate of settling of 8-8-100 mixtures.

were not available, so a report of the experiments made to determine this point is offered as a possible aid to others.

Two sets of experiments were performed, (a) with pure calcium hydroxide to which known quantities of calcium carbonate were added; (b) samples of hydrated lime which upon analysis showed the amount of carbonate desired for experimental purposes. The calcium hydroxide in (a) was prepared by firing hydrated lime until all moisture and carbon dioxide were driven off.

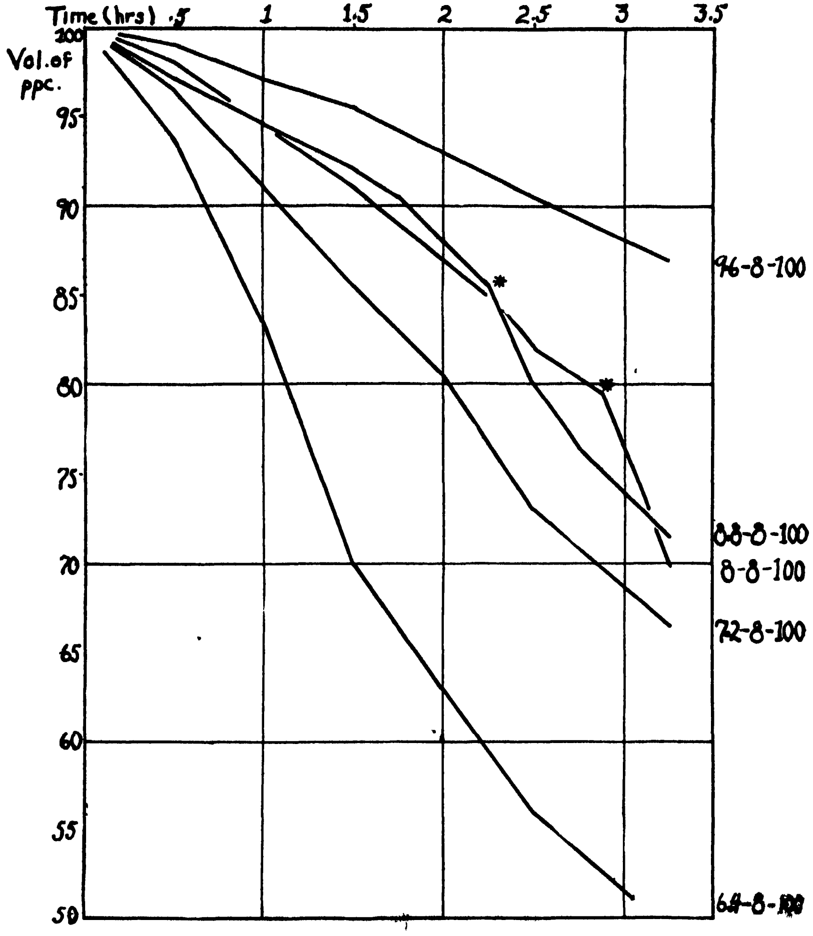

Fig. 2

Comparative rates of settling of mixtures containing various ratios of CaO – $CuSO_4$

* – $Fe(CN)_6^{=}$ added.

The Bordeaux solutions were made as follows: 1.32 gm. finely powdered $Ca(OH)_2$, or a mixture of $Ca(OH)_2$ and $CaCO_3$ of known composition was mixed in a beaker with 50 cc. of water. Fifty cc. of a solution of copper sulfate (31.2 gm. $CuSO_4 \cdot 5H_2O$ to 1 liter) were then prepared and the two solutions combined, thoroughly stirred and then poured into a 100-cc. graduate. The volume of precipitate was then noted at definite intervals of time. The rate of settling, as shown in figure 1, was taken as an indication of the physical qualities of the mixture. The film formation, as seen under the microscope, corresponded to the settling test, but the distinction was less sharp; A and B showed a continuous durable film. The film in C broke early and from then on it lacked from thirty to fifty per cent of being continuous. The difference in color was also quite marked, A to C inclusive being a deep blue; D, pale blue; E, a bluish green; and F, a greenish blue.

The curves in figure 1 show a striking effect of a high carbonate content on the physical properties of the Bordeaux mixture. None of these mixtures showed free copper with the potassium ferro-cyanide test, but the character of the precipitate after standing for two or three hours, color and film structure all showed deterioration. Such proportions of calcium carbonate are possible in hydrated lime that stands for any length of time, as shown by the following data. The samples were small, containing not more than two or three pounds each, but they are an indication of what occurs on the outside of a sack.

TABLE 1.—*Percentage of calcium hydroxide found in samples of hydrated lime*

Container	Original per cent of $Ca(OH)_2$	Date of Analysis	Final per cent of $Ca(OH)_2$
Air-tight tin can	96.9 (January 1st)	February 3d	96.8
Paper bag			
Outer part	96.9 (January 1st)	February 5th	74.4
Inner part	96.9 (January 1st)	February 5th	90.4
Cloth sack			
Outer part	96.9 (January 1st)	February 18th	51.2
Inner part	96.9 (January 1st)	February 16th	96.8

Coarse hydrated lime also hastened the settling rate but was not as detrimental on the whole as a high carbonate content.

Increasing the proportion of copper sulfate retards the settling rate, as shown in figure 2, and may, if necessary, be used to overcome this difficulty.

CONCLUSIONS

The data show that the rate of settling increases almost proportionately to the amount of carbonate and that this should not be greater than twenty per cent and preferably less. Lime containing from thirty to sixty per cent of carbonate makes a very poor type of Bordeaux. The settling rate is also increased by using poorly pulverized lime; the larger the particles, the faster the precipitate settles. The rate of settling is retarded by increasing the proportionate amount of copper sulfate. Artifically prepared and natural occurring carbonated limes produced practically the same physical qualities in the Bordeaux solution.

PHYTOPATHOLOGICAL NOTES

Verticillium wilt of tomato.—While studying the Grand Rapids disease of tomatoes in the field in northern Ohio and in Erie County, Pa., in Sept., 1924, it was found that this disease was occurring in considerable amounts up to 25 per cent in tomato fields, some of which were supposed to have the Fusarium wilt only. One such field of one-half acre showed 90 per cent infection with the combined organisms.

On examination of stems with a hand lens and later in sections under the microscope, both the bacterial and fungus parasites were found to occur in the same field, sometimes in the same plant. As the season was late and the plants far gone, the diseases could not readily be distinguished by casual examination. By cutting the stem across within 6 or 8 inches of the top, however, the differences were clearly evident. In the case of the bacterial disease, the vascular region showed a yellowish discoloration extending into pith and cortex, but no browning. In stems with the fungus disease, on the other hand, a dark brown stain was evident, confined to the narrow vascular ring. In some cases cuts had to be made much lower on the stem before this brown color was evident, but more often it extended to the very tip of the stem.

Specimens of these discolored stems brought to the laboratory for further study and for culturing the fungus have given evidence that the greater part of the fungus wilt was caused by a Verticillium resembling *V. albo-atrum*. Successful infections have been obtained on tomatoes in the hothouse with the Verticillium isolated which was again cultured from the inoculated plants.

That the two fungus diseases of tomato should be confused in the field is not strange since Carpenter[1] states that "The wilt diseases of the several plants brought about by *F. vasinfectum* and *V. albo-atrum* manifest the same symptoms, so that the real cause of the trouble is safely to be determined only by cultural means." Although in England *Verticillium albo-atrum* is a more serious wilt disease of tomatoes than is Fusarium, and although it is also the cause of destructive wilt in numerous plants in America, notably eggplant, potato and okra, no mention has been found in literature of a Verticillium wilt of tomato in the United States. Successful cross-inoculations on tomato with the eggplant strain of *Verticillium albo-atrum* have been made however by Jagger.[2]

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[1] Carpenter, C. W. Wilt diseases of okra and the Verticillium wilt problem. Jour. Agr. Res., 12: 538. 1918.

The demonstration of Verticillium wilt where Fusarium was supposed to be causing the trouble suggests that perhaps this fungus is doing damage in other places in northern tomato fields where cool temperatures would favor its ravages and retard Fusarium.—MARY K. BRYAN.

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[2] Jagger, I. C., and Stewart, V. B. Some Verticillium diseases. Phytopath. 8: 15–19. 1918.

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CERTAIN ASPECTS OF THE VIRUS DISEASES

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INTRODUCTION

Studies of the rosette disease and a foliage mottling of wheat (26, 28) have shown that these disorders behave in many ways like certain of the mosaic diseases. The association of cell inclusions (28) with the diseased wheat plants has naturally led to an inquiry into the literature dealing with cellular pathology, and as the investigations of rosette and cell inclusions have progressed it has become necessary to obtain a knowledge of some of the more general phases of the virus problem as it applies to both plants and animals.[2]

Although it is fully recognized that the virus diseases of man and other animals are independent of those occurring in plants, it is evident that certain aspects of the virus problem are of general biological significance. This appears to be especially true of the viruses themselves and of the pathology of the host cells. The students of animal pathology have spent more time in the study of the viruses and diseased cells than have plant pathologists, and some of the results obtained and theories advanced by the former have suggested the possibility that these may apply directly or indirectly to the virus diseases of plants. The writer intends to consider some of these phases briefly. The animal virus diseases have already been summarized by Wolbach (44), Arkwright (3), Simon (38), and others.

VIRUS DISEASES OF ANIMALS

Although the mosaic of tobacco (4) was the first disease demonstrated to be caused by a filterable virus, it is not surprising that the virus causal agents have received greater attention in their relation to man and the lower animals than to plants. The virus diseases of animals have been studied from many angles, and marked progress has been made, especially in connection with the control of many of them.

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[1] The substance of this report was submitted to the Office of Cereal Investigations and the chief of the Bureau of Plant Industry on March 10, 1924.

[2] Through the kindness of Dr. R. W. Hegner, Dr. C. E. Simon, and others at the School of Hygiene and Public Health, Johns Hopkins University, the writer was enabled to study their splendid collection of cell inclusions associated with certain virus diseases of man and the lower animals and to get in touch with many of the virus-disease problems.

At the present time there appear to be some fifty or more of these diseases, and in most of them their viruses pass some of the standard types of earthen or porcelain filters. Numerous data have been obtained which show that many of these viruses differ greatly in their ability to pass filters of different porosities. These differences have been attributed largely to supposed differences between the sizes of the infective particles. However, it has been pointed out by several workers that other factors, such as the possible plasticity of the particles, the concentration of the virus, and the nature of the fluid containing the virus, tend to influence filterability.

Many of the animal viruses have also been studied to determine the influence of various chemicals, disinfectants, temperatures, and other factors on their infective properties. Most of the animal viruses are inactivated at temperatures between 50° and 60° C. Low temperatures, freezing and thawing, and desiccation have little detrimental effect on many of them. A good many of the viruses are surprisingly resistant to glycerin, alcohol, and disinfectants.

In addition to the study of the viruses themselves, considerable attention has been devoted to the diseased cells of the various hosts. In many cases certain cells of the affected animals show fairly definite inclusions, which have been studied by many investigators and are interpreted in several ways. These interesting bodies are associated with 20 or more of the virus diseases, and it is thought that they may occur in association with a still greater number of these maladies. In some cases there is a question as to the constancy of the occurrence of inclusions with a given disease; but in many instances they seem to be rather definitely associated, even to the extent that they may be used as a basis for diagnosis, as in the case of the Negri bodies of rabies. Many workers believe that the presence of inclusions in the cells of an animal affected by an unknown malady gives good reason for studying the disease according to virus methods. On the other hand, there are those who scout this idea.

Several distinct types and forms of cell inclusions have been found associated with these diseases, and Lipschütz (24) has classified them on the basis of their relation to the contents of the cell, dividing them into three groups as follows:

(1) Cytooikon group: Inclusions present in the cytoplasm.

(2) Karyooikon group: Inclusions present in the nucleus.

(3) Cytokaryooikon group: Inclusions present in the cytoplasm and the nucleus.

The exact nature of cell inclusions has been a much debated question. Calkins (8) conducted morphological studies on the Guarnieri bodies associated with smallpox (variola) and considered them protozoa and the parasites causing the disease. Negri (31), who discovered and studied the cell inclusions associated with rabies, also concluded that they are protozoan parasites and the cause of the disease. These workers observed minute granules in association with the larger inclusions and considered these the regenerative forms (gemmules of Calkins) produced by the larger body, or "protozoan."

Halberstaedter and Prowazek (16) studied the morphology of the cell inclusions and minute granules associated with the trachoma disease of the human eye and concluded that the inclusions themselves are not organisms at all but merely products secreted by the nuclei of the diseased cells. They[3] believed that the minute granules are true organisms and the etiological agents, that these "organisms" stimulate the cell nucleus to extrude nucleolar substance ("plastin") into the cytoplasm, and that this material forms the inclusions which serve as "mantles" for the minute "organisms." They called these supposed organisms "Chalamydozoa," on account of this apparent mantle, and applied their theory to the cell inclusions and microscopic granules associated with other virus diseases of animals. Lipschütz (23) named the minute granules or supposed organisms "Strongyloplasmen." He and other workers believe that these granules pass through bacteria-proof filters.

Attempts to cultivate the animal viruses *in vitro* have been numerous. In some cases positive results appear conclusive, and at the present time Arkwright (3) considers that four "viruses" (of pleuropneumonia of cattle, avian diphtheria, influenza, and poliomyelitis) have been cultured successfully. Noguchi (32, 33) applied culture methods to the study of the virus of rabies and that of trachoma. In his work on the rabies virus he states that the "parasite" grew in culture, and his report indicates that he obtained stages in the culture solution which were similar to the Negri bodies and the minute granules found in certain cells of rabid animals. He claims to have obtained the typical symptoms of rabies in animals which he inoculated with culture material containing these "granular, pleomorphic or nucleated bodies." The photographic evidence which Noguchi produced on this point indicates that his culture solutions did contain structures very similar to Negri bodies. In the light of this behavior Noguchi considered that the Negri-like or "nucleated bodies" are independent organisms which exhibit the appearance of protozoa. He does not mention any controls for these experiments. Noguchi (33) also claims to have cultured an organism from trachoma. He reports that these organisms showed morphological features similar to typical trachoma inclusions, and his photographic evidence seems to bear out his claim. However, these cultures did not produce the disease when used in inoculation experiments.

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[3] It is generally accepted that von Prowazek developed this and the Chlamydozoan theory.

Moon (30) attempted to cultivate *in vitro* the virus of rabies, and although he makes no definite claims he considers that he obtained an increase in the amount of virus. He reports that bodies similar to Negri bodies developed in some of his cultures. He points out, however, that in certain cases degenerated brain tissue in his culture tubes might cause cell nuclei to be confused with Negri bodies. Poor and Steinhardt (36) also attempted to cultivate the virus of rabies but report negative results. The methods employed by these workers differed from those of Noguchi and Moon. Steinhardt and her co-workers (40, 41) report the culture of the virus of cowpox (vaccinia) in tissue cultures, and although they claim to have produced the disease from the third subculture from the original planting they did not obtain cell inclusions. They did find minute granules which were similar to those in diseased cells, but these were in their controls also. Several other workers have attempted to cultivate the viruses of rabies, trachoma, and vaccinia but without success. At the present time many investigators consider that these as well as many other viruses have not yet been successfully cultured *in vitro*.

The theories which have been advanced concerning cell inclusions and the small granules associated with certain virus diseases have met with rather wide acceptance for a time among certain groups of workers. In general, it appears that the ideas of Prowazek and Lipschütz are now entertained by a great many investigators. Others, however, are holding open minds as to the nature of cell inclusions and the microscopic granules until more experimental data are available.

VIRUS DISEASES OF PLANTS

The main features of the virus diseases of plants have already been summarized by Dickson (9), Butler (7), and others; therefore it is not necessary to go into the general subject fully at this time. There are several distinct types of infectious diseases of unknown cause which are characterized by chlorosis and several other types of symptoms, such as foliage distortions, blight, excessive green coloration, arrested growth, proliferation, internal lesions, and galls; and some of these diseases may manifest nearly all of these symptoms in varying degrees. Generally, they seem to be very restricted in host range, as is the case with the most virus diseases of animals. The mosaics are an exception, however, as they occur in a wide range of hosts among the monocotyledonous and dicotyledonous plants, producing strikingly similar chlorotic-mottling symptoms in all. In this respect the mosaics seem to hold a position among plant virus diseases somewhat similar to that of rabies and certain poxes among the virus diseases of animals. According to Stimson (42) rabies will develop and run its course in practically all mammals. He states that fowls also are susceptible to inoculation, and that frogs and tortoises are sometimes refractory to the disease. In rabies, however, a single virus is involved; at least studies of its properties do not indicate that different viruses exist. Several of the pox diseases occurring in some mammals produce their similar but somewhat modified symptoms on different species, but in these cases it appears that different viruses are involved.

Of all the virus diseases of plants, the mosaics have received the most attention, particularly the mosaic of tobacco, partly because it was the first disease demonstrated to be caused by a filterable virus; but more probably because it is very readily transmitted by artificial means and the virus is highly resistant. Relatively little work has been done on the properties of plant viruses, and that which has been done deals largely with the viruses of the mosaics, especially tobacco mosaic. This virus is very resistant to desiccation, temperature, various chemicals, and disinfectants. Allard (1) found that it was not inactivated when subjected to temperatures of 85° and 90° C. for 10 and 5 minutes, respectively. On the other hand, the virus of cucumber mosaic (11) is inactivated in 10 minutes at temperatures above 70° C. This virus is also less resistant to desiccation and to various chemicals than that of tobacco mosaic.

It is fully recognized that certain difficulties are encountered when an attempt is made to interpret data obtained from property studies made on the mosaic viruses. These substances have not been isolated in a pure state, and therefore, for the present at least, they must be studied in association with the host fluids. Plant juices are complex in nature, and it is not known just how much influence they may exert on the behavior of a given virus when subjected to different treatments. However, even though it is granted that this situation constitutes an obstacle and that the present methods of carrying on property studies may be considered rather crude, it still appears that there is complete justification for investigating the properties of the viruses. Undoubtedly such studies will lead to the development of new and more satisfactory methods and to new interpretations.

The property of inter-host transmission of the mosaic viruses has been studied to a considerable extent by Doolittle (11, 12, 13), Walker (43), Elmer (14), Brandes and Klaphaak (6), and others, and very significant results have been obtained. Doolittle and Walker have found by direct inoculation methods that the virus of cucumber mosaic will produce mosaic in a number of dicotyledonous hosts. Some of these hosts are closely related to the cucumber, whereas others are widely separated in relationship. Brandes and Klaphaak carried on direct cross-inoculation studies with the virus of sugar cane mosaic and found that it produces the disease on a large number of grasses but in no case on species distantly related to the cane plant. These and similar results obtained by other investigators have naturally led to the belief that there are probably several mosaic viruses, each of which may have a different host range.

In addition to making direct cross inoculations Doolittle and Walker (13) obtained infections through intermediate or bridging hosts which had been impossible to secure by the direct method. They found that the mosaic of cucumber may be transmitted to tobacco provided the virus is first passed through the pepper plant, whereas their investigations show that this cross inoculation is not successful if made directly. Elmer (14) also conducted many cross-inoculation experiments and obtained certain results which are very striking. He reports that the mosaic of sugar cane is transmitted to tobacco when the virus is treated with acetone. Without the acetone treatment he claims negative results. He also reports infection from cross inoculations between other hosts of rather distant relationship.

Some workers have failed to verify certain mosaic infections from cross inoculations which others have reported. Although some of these discrepancies appear to be caused by faulty methods, it is possible that certain of these apparent inconsistencies may be explained on the basis of an overlapping of the host ranges of viruses which are not identical in all respects.

Although the cross-inoculation studies throw considerable light on host ranges and on the differences between mosaic viruses, it appears that these studies should be coordinated with the study of other properties of viruses.

In cucumber mosaic (11) the virus is presumably inactivated when subjected to temperatures above 70° C. for 10 minutes, whereas the virus of tobacco mosaic (1) is not inactivated when held at 85° C. for the same length of time. The cucumber-mosaic virus is also less resistant to drying and retains its virulence for a shorter period than the virus of tobacco mosaic. The question now arises as to the stability of the thermal "death" point and other properties of a given virus when it is subjected to different treatments and to a direct or bridging host cross. Will the thermal "death" point of the cucumber-mosaic virus remain fixed after it passes through the pepper plant and gets into the tobacco plant, or will this property change and approach the thermal "death" point of the tobacco-mosaic virus? Will its reaction to drying or to chemicals and its retention of virulence be changed to any extent by this cross infection? The same general questions also arise concerning the sugar cane-acetone-tobacco cross infection reported by Elmer (14). Obviously such studies necessitate systematic investigations of the properties of the viruses in conjunction with cross inoculations. Although this procedure entails considerable effort, it is evident that the data from such combined studies will be of much greater value than those obtained when no attempt is made to coordinate property studies with cross-inoculation work.

It would seem that a knowledge of the stability or instability of the properties of viruses after passage through various hosts or after subjection to other treatments will give us a better idea as to the possibility that all mosaics are due to a single virus or to several different ones. Such information may also throw some light on the nature of the viruses.

Several attempts have been made to cultivate plant viruses *in vitro,* and certain investigators claim to have obtained interesting results. Bewley (5) reports that he obtained an unusual type of growth from the virus of tomato mosaic in culture tubes, but his inoculations were not completed at the time he published and it is impossible to draw final conclusions regarding his work. McWhorter (29) applied tissue-culture methods in his studies of the cell inclusions associated with the Fiji disease of sugar cane and claims to have observed the germination of the bodies. He concludes that they are amœbæ.[4]

Recently the causal phase of the plant-virus problem has been attacked vigorously by means of histological and cytological methods. In several cases it seems that diseased cells show striking similarities to those in many animals attacked by virus diseases; in fact, this similarity appears so marked that it led Palm (34) to consider that the cell inclusions and microscopic granules associated with tobacco mosaic are Chlamydozoa-Strongyloplasmen.

CELL INCLUSIONS

As shown in table 1, cell inclusions are known to be associated with a few infectious diseases of plants, and in most instances these particular diseases are known to be caused by viruses. However, in other cases the causes are of a less definite nature. So far as known, all of these inclusions occur in the cell cytoplasm. None has been found in the nucleus, as have some of the inclusions in diseased animal cells.

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[4] Since the preparation of the present paper Olitsky (Jour. Exp. Med., Vol. 41, No. 1, 1925) reported the successful increase of the causal agent of tobacco and tomato mosaic in fluid from tomato plants. This is the most convincing paper which has yet appeared on this phase of the plant virus problem. Regardless of the interpretation which may be placed on this increase of the virus, the demonstration that a plant virus apparently increases *in vitro* is significant in that it paves the way for the development of new methods for studying the mosaic problems. The results encourage the development of tissue culture methods and they stimulate one to make a systematic attempt to discover some green alga which may be susceptible to a mosaic virus. Such a combination would facilitate the matter of obtaining much direct microscopic evidence on many obscure processes and also enable the accumulation of much data in a simpler manner than is now possible.

Iwanowski (17) appears to have found the first cell inclusion associated with a definite virus disease of plants. In 1902 he reported these bodies in the cells of the light-green areas of mosaic tobacco leaves. In these same cells he also found many small granules which he thought were bacteria. He interpreted the large inclusions as products of degeneration. In 1922 Palm (34) also found inclusions and granules in the cells of mosaic tobacco leaves, and he came to the conclusion that they were the Chlamydozoa of Prowazek (the Strongyloplasmen of Lipschütz). In 1924 Rawlins and Johnson (37) and Goldstein (15) also confirmed Iwanowski's observation.

TABLE 1.—*List of host plants and diseases with which cell inclusions have been found to be associated.*

Disease	Host	Authority[1]
Brunissure	Grape	35
Mosaic	Tobacco	15, 17, 34, 37
Fiji	Sugar cane	22, 25, 29
Mosaic	Corn	18
"	*Hippeastrum equestri*	19, 20
"	Sugar cane	21
"	Potato	39
Rosette and mosaic mottling	Wheat	28
Mosaic	*Hippeastrum johnsonii*	27

[1] See "Literature cited," p. 200.

As in the case of animal-cell inclusions, many theories are being advanced concerning the nature of inclusions in plant cells. Lyon (25) was inclined to think the bodies in the gall cells associated with Fiji disease are organisms, and McWhorter (29) claims definitely that they are amœbæ. Kunkel (22) inclines to the belief that they are organisms on account of cytological evidence which indicates that they grow and divide in the cell and that the inclusions associated with the mosaics of corn (18) and *Hippeastrum equestri* (19) are organisms. Rawlins and Johnson (37) do not attempt to interpret the cell inclusions occurring in mosaic tobacco. Goldstein (15) thinks they are not secretion or degeneration products arising from or in the neighborhood of the nucleus. She reports observations dealing with the movement of these cell inclusions which are apparently considered suggestive that the bodies may be independent organisms. The writer has observed this phenomenon many times in the cell inclusions associated with wheat rosette. However, it has always been very difficult to differentiate with certainty between what might be a pushing out or drawing in of the periphery of the body, suggesting pseudopodia, and the rolling and tumbling movements of irregularly shaped rigid bodies when circulating about in the streaming cytoplasm. Inclusions such as those illustrated by McKinney, Eckerson, and Webb (28, pl. 6, fig. 5; pl. 7, figs. 6 and 10; pl. 8, figs. 2, 4 and 10) may assume very curious and irregular shapes when moving in the streaming cytoplasm. Smith (39), who observed amœba-like cell inclusions associated with potato mosaic, thinks these bodies are degeneration products, probably from the nucleus. Politis (35), who has done the most recent work on the brown corpuscles in the cells of the grapevine affected by the brunissure disease, thinks that these cell inclusions are not organisms, as others have believed, but are produced by a transformation of the granular mitochondria in the diseased cells.

At the time the writer published his first paper on the rosette disease (26), the cell inclusions occurring in wheat were briefly compared with those present in certain other plants. At that time the comparisons were based on published descriptions and illustrations. Since then it has been possible to study some of the inclusions occurring in plants other than wheat. Although extensive comparisons have not been made under different sets of conditions, preliminary observations indicate that some of these inclusions differ in their general structure and morphology, and there is a suggestion that all of them may not originate in the same manner. Also, their general relations to the host cells seem to differ to a considerable degree. In general, the inclusions associated with tobacco and potato mosaics, the Fiji disease of sugar cane, and the rosette disease of wheat resemble each other in form and structure, differing somewhat in their relations to the host cells. In a few cases some of these inclusions appear to be made up of what appears to be a rather finely granular matrix. However, most of the bodies possess a matrix which is strikingly homogeneous in nature. Except in the very early stages of development, the matrix of all these inclusions contains vacuoles which vary in number and size. In some cases large granules may be present in the matrix or in the vacuoles, and occasionally the large vacuoles appear to be filled with a fine granular substance. It is not uncommon to find inclusions in this group which appear to be spheres. The outer matrix, or shell, of such a sphere will contain many very small vacuoles, and there may be one large central vacuole or several smaller ones located in the interior. All of these inclusions appear to be surrounded by a membrane and to consist of protoplasm. They are strikingly similar to certain forms of Negri and Guarnieri bodies associated with rabies and smallpox, respectively.

The cell inclusions associated with corn, Hippeastrum, and sugar cane mosaic present a somewhat different appearance from the above-mentioned inclusions. Many of them seem to be made up of compactly arranged rather large, independent granules, sometimes around "vacuoles" or unoccupied areas. In many cases the writer has caused the separation of these granules by pressure and has seen no evidence that these bodies are surrounded by a membrane. In some ways these inclusions resemble the Prowazek bodies which are associated with the trachoma disease of the human eye. They are not so suggestive of protoplasm as the bodies mentioned in the previous paragraph. In the studies on the inclusions associated with *Hippeastrum johnsonii* (27) the writer finds that many of the host nuclei are shrunken and small in cells which contain inclusion bodies. Kunkel (18) observed this in corn. This condition has not been noted among nuclei in wheat cells containing inclusions.

The writer is not yet in a position to interpret the nature of the cell inclusions he has studied most intensively in the wheat plant. As in the case of the Fiji bodies (22) they increase in size, and in the few instances where two bodies have been noted in one cell there has been evidence of division; however, these phenomena do not seem to lend any more support to the theory that the bodies are independent organisms than that they may be protoplasmic aggregates arising within the cell. If these and many other cell inclusions and cell granules are independent organisms it seems rather doubtful whether the case can be proved conclusively by the present cytological methods and optical facilities. If they are organisms, the fact can best be determined through culture and inoculation studies in combination with cytological methods.

Although the cell inclusions associated with some of the virus diseases of plants resemble those in certain animal virus diseases, it is questionable whether the interpretations which have been developed on the animal side regarding the nature of inclusions and microscopic granules should be accepted too freely by plant investigators; and although several of the methods which have been used to study animal inclusions may be applicable to the study of these bodies in plants, it appears from preliminary investigations that additional methods must be developed by plant workers. Giemsa's method of staining has been used widely in the study of animal-cell inclusions, and the conclusions of several workers are based to a large extent on results obtained with this stain. However, the writer's experience with this stain in plant tissues has not been very satisfactory. After more experience with it dependable results may be obtained, but at present the writer is inclined to agree with Dobell (10) that the value of this method has probably been somewhat overestimated.

From the observations made thus far it seems thoroughly worth while to determine the extent of the association of cell inclusions with plant-virus diseases, especially in the case of the mosaics. The writer's experience indicates that cell inclusions may not be associated with all of the mosaics; or, if so, they may not be abundant in all cases. To date, it has not been possible for him to find definite inclusion bodies associated with the mosaics of cucumber, raspberry, or sweetclover. However, these negative findings can not be considered as final, as he has frequently had difficulty in finding very many cell inclusions in some of the mosaic corn, sugar cane, and Hippeastrum grown in this country. Had it not been known that inclusions occur in these hosts, doubtless they would have been overlooked in some of the material examined. It may be that the range of favorable environmental conditions for the occurrence of cell inclusions in some hosts is more limited than that for typical mosaic mottling. It would be of great interest to know something of the inclusion phenomenon which exists in the sugar cane-tobacco mosaic cross which Elmer (14) claims to have obtained. Cell inclusions are associated with both of these diseases, and as these particular inclusions seem to show different characteristics it is important to know whether the cane virus produces the cane type or the tobacco type of inclusion in the tobacco plant. Likewise it is important to determine the behavior of inclusions in the cucumber-pepper-tobacco cross. Such observations should bring out correlations between cell inclusions and the hosts or the viruses which will throw additional light on the probable nature of inclusions and on the specificity of viruses.

One of the striking results which comes out of a general study of the virus problem is that no single lead or line of attack stands out as the one most likely to lead to the determination of the exact nature of the viruses. On every hand the evidence shows that no matter which single line of attack is followed, the limitations of present methods seem to prevent the attainment of the facts most desired. To the writer it appears that there is complete justification for piling up indirect as well as direct evidence on all phases of the virus problem. It appears also that many of the single lines of study should be coordinated and new experiments should be carried out in order that we may obtain a better idea as to the adequacy of some of the experimental methods in use and so that many of the theories which have been advanced may be considered from new angles.

A systematic study should be made of the environmental factors which may influence the various phases of these diseases. Soil and air temperature studies have been carried out with a few hosts and it is interesting to note that the mosaic diseases appear to be most injurious at temperatures which are seemingly most favorable to the development of the host plants.

This general relationship seems to be rather unusual and one is naturally led to inquire as to its possible significance. It would be of considerable interest to know how general this relationship may be among the mosaics and other virus diseases. With the increasing tendency on the part of workers to give consideration to the idea that some component of the protoplasm of the healthy host may become the disease-producing agent or virus, it appears that more time should be devoted to the study of healthy plants grown under various environmental conditions. In considering this theory it would seem that some irregularity in the hosts' environment must play an important part in causing the first upset which results in the development of a disease producing virus, and it appears that the only way to prove the theory beyond doubt is to determine the conditions which may cause this supposed first upset in the healthy host. The effect of ultra-violet light on the vitamin content of many foods thought to be devoid of certain of these substances is a case not wholly unlike the one under consideration.

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QUANTITATIVE STUDIES ON THE EFFICIENCY OF FUNGICIDES

JEAN MACINNES[1]

WITH NINE FIGURES IN THE TEXT

Considerable information has accumulated with reference to the efficiency of various substances both for disinfection and for the control of plant diseases. The results obtained have been largely empirical, however; and, although attempts have been made to define the factors which make one substance an effective killing agent and another a poor one, only a few investigators have made use of quantitative physical and chemical measurements for this purpose. Among these investigators may be mentioned Osterhout (4), Brooks (1), and Shearer (2, 3). In the experiments described in this paper, such measurements were made with the hope of obtaining some definite information regarding the subject of toxicity and also with the hope of developing a rapid method of comparing the effectiveness of fungicides.

THE APPARATUS

The apparatus which was adopted for making the measurements consisted primarily of three glass cells, a diagrammatic representation of one of which is given in figure 1. One electrode (F) was fused into the base of the cell, the other (C) being inserted from above. The organism was placed between two perforated hard-rubber disks (D and E), and inside a cavity made by disks of soft-rubber (H). Fresh solution was constantly perfused through the organism by means of the tube (N) entering at the base of the cell.

The three cells were fastened securely on a stand and remained there throughout each experiment. The upper electrodes were fastened on the stand in such a manner that they could always be replaced in exactly the same position in relation to the lower electrodes. They were also arranged so that the distance between them and the lower electrodes was approximately the same for all three cells. In this way it was possible to have the initial resistance of the three cells, before the organism was placed in them, very nearly the same.

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[1] The author wishes to express her appreciation of the assistance and encouragement given her by Professor Prescott, of the Department of Biology and Public Health of the Massachusetts Institute of Technology, and Professor Osterhout, of the Department of Botany of Harvard University.

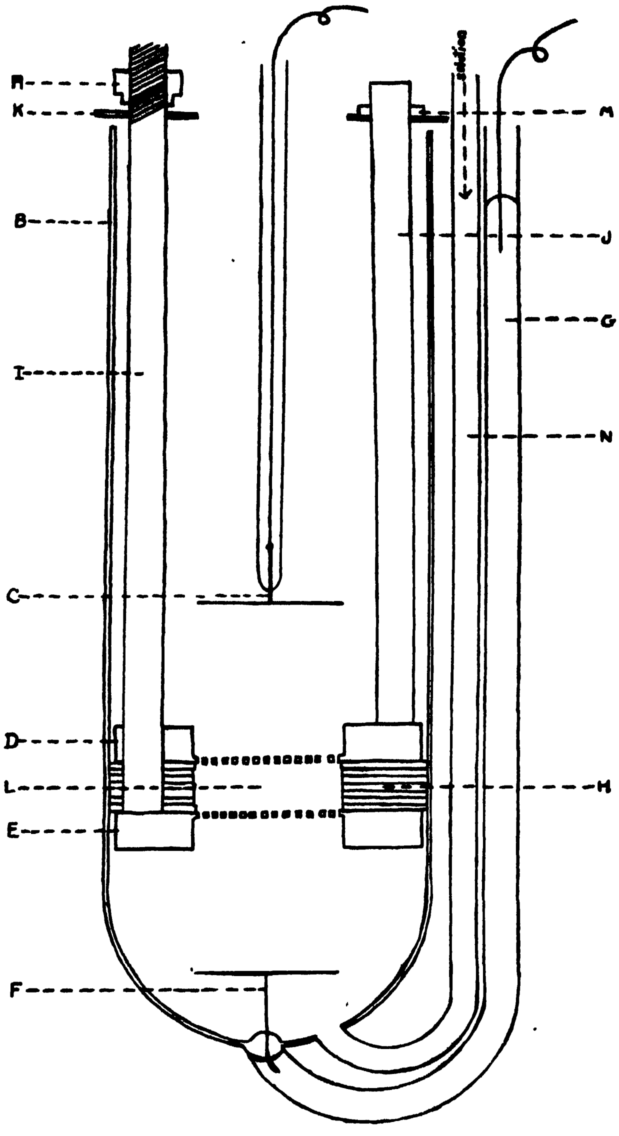

Fig. 1

FIG. 1. Diagrammatic representation of one of the cells in which the conductivity measurements were made. A. Bolt for adjusting the position of the disks. B. Wall of the glass cell. C. Upper electrode. D. Upper hard-rubber disk. Note perforations for passage of the current and the solution. E. Lower hard-rubber disk. F. Lower electrode—fused into base of cell. G. Tube filled with mercury for making connection with

The position of the hard-rubber disks in relation to each other had to be controlled very carefully. In order to do this, three rods of hard-rubber were attached to the upper side of each, the rods of the lower disk (see fig. 1) (E) passing through the upper (D) in such a manner that they alternated with those of the upper. All six rods terminated at the top of the cell, but those coming from the upper disk were fastened to a metal ring (K), and the others passed through holes in the ring and were threaded for bolts (A).

As it was impossible to fit these hard-rubber disks so tightly that none of the current passed between the edge of the disk and the glass, similar disks of soft-rubber (H) were made. They were slightly greater in diameter than the hard-rubber ones but were less than an eighth of an inch in thickness, and, instead of being perforated, had holes three quarters of an inch in diameter cut in their center. Seven such disks were superimposed on the lower hard-rubber disk, the rods from the latter passing through holes in the soft rubber made for that purpose. When the upper disk was put in and the bolts screwed into place, the soft-rubber expanded and pressed against the cell sufficiently to prevent the passage of current through that part of the cell, a cavity of about a half an inch in depth and three quarters of an inch in diameter being formed in the center. The organism was stuffed into this cavity.

The solution which entered at the base of the cell through the tube (N) passed up through the perforations in the lower disk, through the organism, through the upper disk, and was drawn off by means of suction. The soft-rubber disks prevented it from passing between the walls of the cell and the disks. The solution, before entering the cell, was conducted through the water bath in glass tubes from a large bottle held about two feet above the apparatus. A constant rate of flow of the solution was adopted which was shown to be sufficient to keep the resistance of the solution the same throughout the time that the experiments were being carried out.

The temperature of the water bath in which the whole apparatus was immersed was 25 ± .1° C. Figure 2 is a diagrammatic representation of the whole apparatus.

lower electrode. H. Soft-rubber disks. I. Rod of hard-rubber coming from lower hard-rubber disk. J. Rod from upper hard-rubber disk. K. Metal ring through which the rods pass and upon which the bolt (A) presses when the disks are in position. L. Cavity in which the organism is stuffed. M. Bolt for holding metal ring to the rods which come from the upper disk. N. Tube through which the solution passes before it perfuses through the organism.

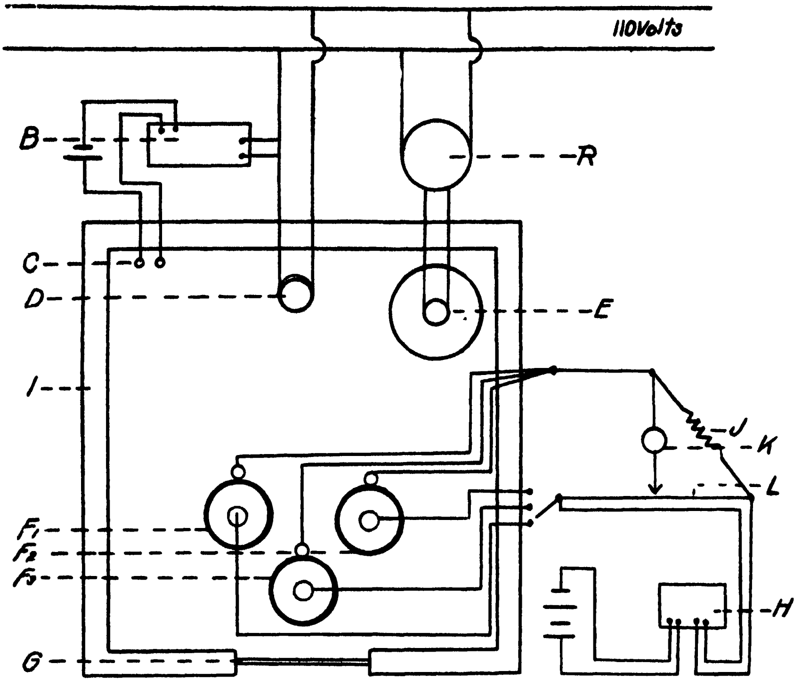

FIG. 2. A diagrammatic representation of the whole apparatus. R. Motor for running the stirrer. B. Relay for controlling the lamp which heated the water bath. C. Mercury thermo-regulator. D. The lamp which heated the water bath. E. The stirrer. F_1, F_2, F_3. The glass cells with their connections to the bridge. G. Window in the tank. H. Buzzer. I. The tank which held the water for the water bath. J. Known resistance on the bridge. L. The slide wire on the bridge. K. The telephone receivers.

THE ORGANISM

The organism used in all of the experiments here to be described was *Aspergillus niger.*

CULTURE MEDIA AND METHOD OF MAKING CULTURES

The medium in which the organism was grown was essentially that which is known as Czapek's solution. It was used four times as strong as recommended by Czapek, however, as it was necessary in these experiments to have a solution of moderately low resistance. The spores of the organism were produced on slants of potato dextrose agar and then inoculated into flasks of 750 cc. capacity containing 100 cc. of the modified Czapek's solution. After being at a constant temperature of 24° C. for about three days, an even growth of the organism was obtained on the surface of the medium.

THE TOXIC SOLUTIONS

The killing agents used in these experiments were mercuric chloride, copper sulphate, and formaldehyde. These substances were added directly to the modified Czapek's solution, the same as was used for growing the organism except that the KH_2PO_4 was omitted.

THE PROCEDURE

Material, grown as described above, was carefully removed from the flasks in which it had grown in such a manner as to prevent tearing or breaking. Each sheet of the mycelium was then cut into three equal parts, and one of each, including some of the culture solution in which the fungus had grown, was immersed in the liquid in one of three suction flasks and the whole put under suction for about five minutes in order to remove a large part of the air held between the cells. In this way, each cell was supplied with an equal amount of exactly the same material.

In order to determine the resistance of the cell without the presence of the organism, the cell was filled with the solution to be used in the experiment, the upper disks put in, the bolts screwed down, and the upper electrode set in place. When the soft-rubber disks pressed against the walls sufficiently to prevent any of the current from passing through that part of the cell, a maximum resistance was obtained. A little experience made it possible to determine this point merely by noting the tightness of the screws.

When the resistance of the solution had been measured, the upper disk was removed and the organism was stuffed gently into the cavity made by the soft-rubber disks. After replacing the upper disk and setting the electrode in place, the resistance of the whole was taken. Perfusion was started at once.

The organism was put directly into the killing agent and the initial resistance obtained considered to be the resistance of the normal organism before it had been affected by the toxic substance. In calculating the results and plotting the curves, this figure was called 100 per cent and the percentage rise or drop in resistance obtained, due to the action of the killing agent, was based on this figure.

CONDUCTIVITY EXPERIMENTS

Before it was possible to carry out the experiments by which the change in conductivity due to the various killing agents could be compared, it was necessary to make a number of preliminary experiments. It was necessary to know, for instance, the changes which might take place in the organism without the presence of the killing agent. Experiments were carried out in exactly the same manner as in all the other experiments, except that the modified Czapek's solution alone was used. Under these conditions, the resistance of the organism remained constant, often as long as three hours. Owing to the accumulation of carbon dioxide produced by the organism, however, the resistance of the organism eventually rose rapidly and never dropped again to the original amount. This fact made it impossible to compare the normal organism directly with that treated with the toxic substance. As these results were totally different from those obtained when the toxic substance was present, however, it was not necessary in this case to base the comparisons on the normal.

It was found that the initial resistance of the organism from one day to the next varied considerably, owing, partly at least, to the fact that it was impossible to inoculate the solutions with exactly the same number of

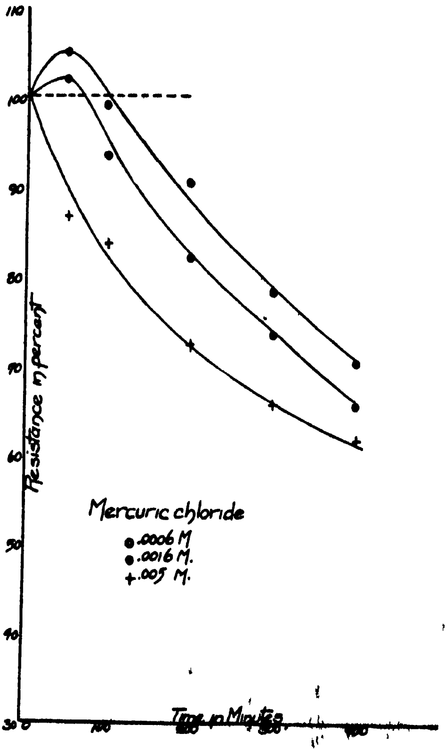

FIG. 3

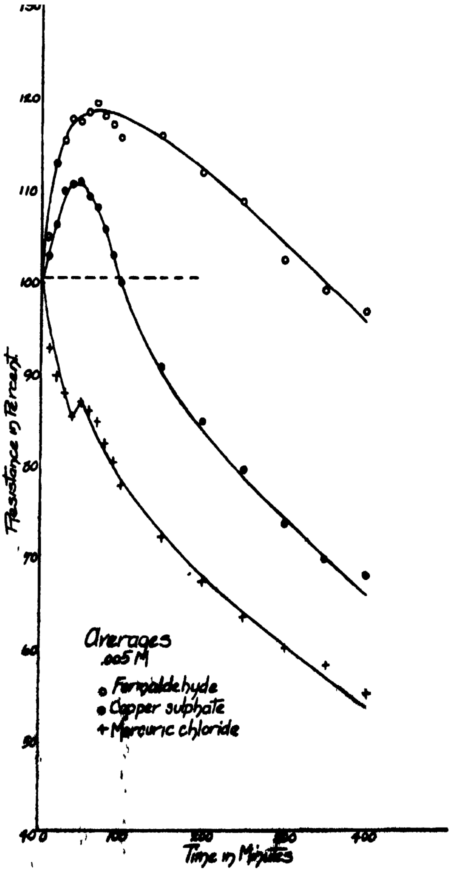

FIG. 4

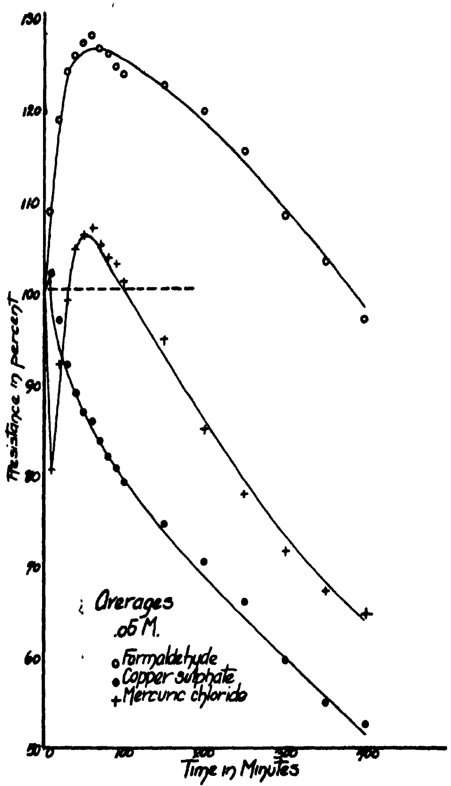

Fig. 5

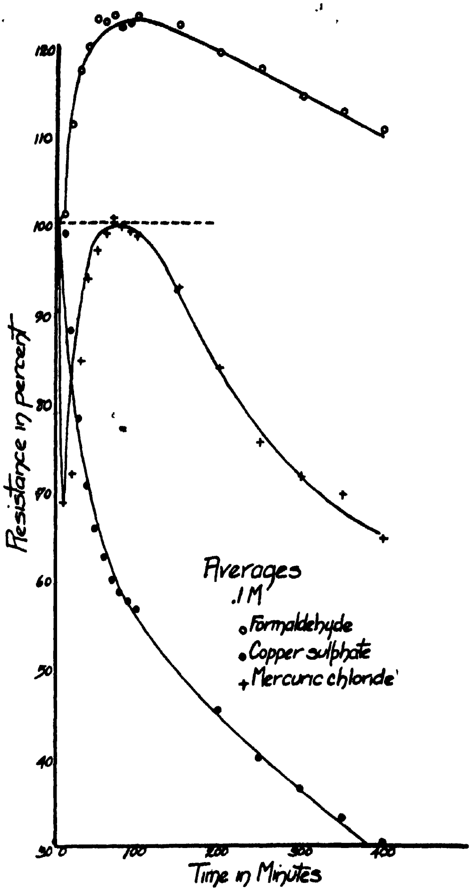

Fig. 6

spores each day. This caused a variation in the quantity of material which developed from day to day, and consequently in the quantity of material which was put into the cells. As the results were all used on a basis of percentage of the original resistance, however, these variations were not of great importance.

In order to determine the extent to which the resistance of the living organism might be changed upon death, typical samples were killed by heat and by the use of copper sulphate. In both cases, the resistance after death was found to be about 25 per cent of what it was when the organism was living. Later experiments indicated that, when it was killed with formaldehyde, the drop was not so great.

When material which had been killed was subsequently treated with the killing solutions in the same manner as the living organism was treated, no such changes as occurred in the latter case were ever obtained. Such changes in resistance as occur with the living organism, therefore, must be a function of the living cell.

With these factors in mind, and after proving that the three cells could be counted on to give comparable results, a series of experiments were conducted in which different concentrations of mercuric chloride were compared. The results are given in figure 3. It is evident that an increase in concentration of the killing agent increases the speed with which the resistance of the organism changes.

Several series of experiments were then carried out in which equal concentrations of the three killing agents were compared, each cell being supplied with an equal amount of identical material, the only variation being that of the toxic substance. In one series, the concentration was .005 M., and later .05 and .10 M. were made use of. The results are given in figures 4, 5, and 6. These same results are made use of in figures 7, 8, and 9, in which the different concentrations of the three toxic substances are compared.

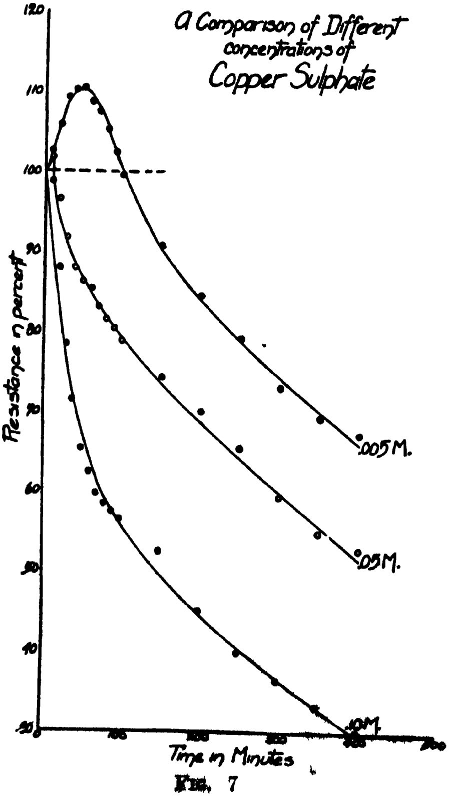

Fig. 7

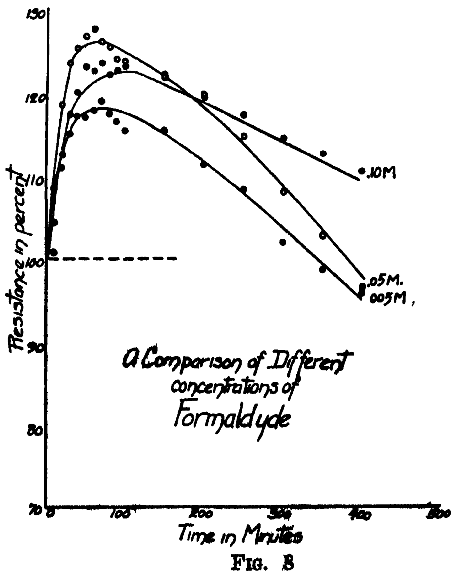

Fig. 8

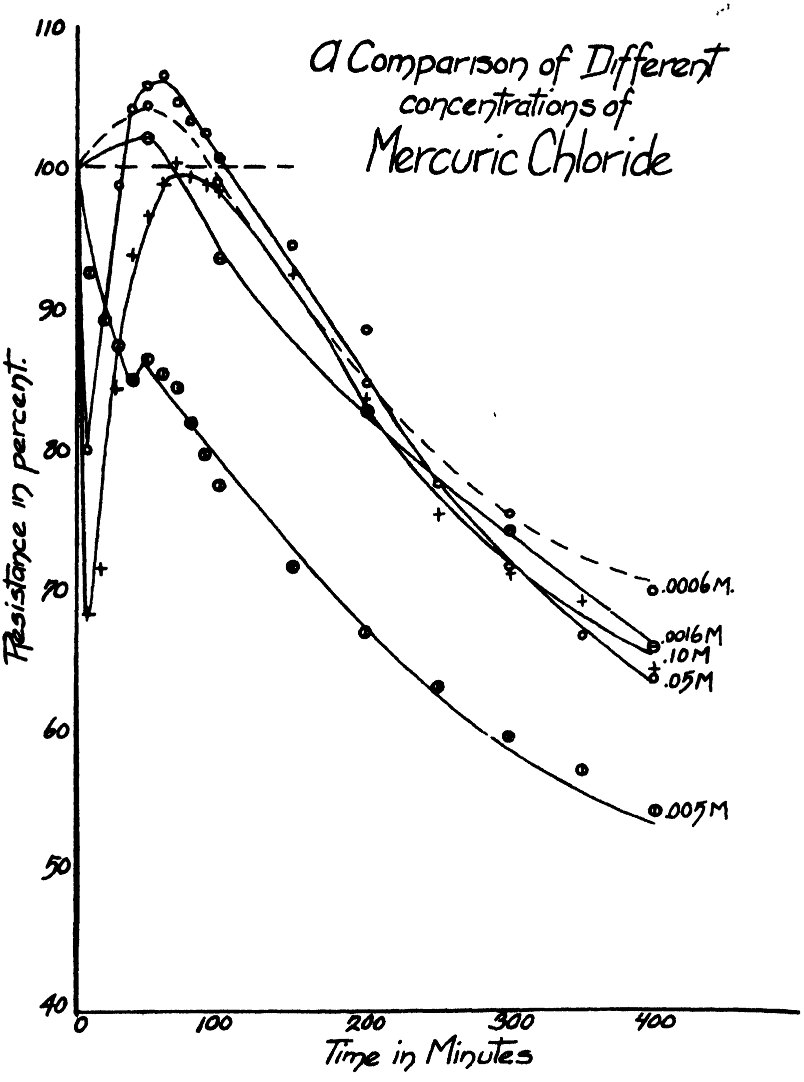

FIG. 9

An attempt was made to find out whether a direct relation could be found between the length of exposure necessary to prevent growth of the organism and the curves obtained for the three killing agents. It was found that mercuric chloride was much more effective in preventing growth of the fungus than either formaldehyde or copper sulphate; and, of the latter, formaldehyde was more effective than copper sulphate. No growth was obtained when the organism had been exposed to mercuric chloride longer than one minute, when the concentration was only .0006 M. The material withstood copper sulphate of .10 M. for more than 200 minutes, but material treated with formaldehyde did not grow if it had been exposed more than 10 minutes to this same concentration.

DISCUSSION

From an examination of the results obtained it is evident that, if electrical conductivity is a measure of the death process, the whole problem is very complicated and that the changes taking place as death is brought about vary with the different ways of killing. It is quite probable that more than one process is being measured. However, a number of definite relationships hold true, and it is fairly safe to conclude that some notion of the relative efficiency of the toxic agents for fungi may be obtained by measuring the changes in electrical resistance.

The results with copper sulphate (Fig. 7) show quite clearly that a direct relationship exists between concentration and changes in resistance. The rate of decrease in resistance with .10 M. copper sulphate is much greater than with .05 M. and very much greater than with .005 M. As a strong solution of copper sulphate would be expected to kill more rapidly than a weak one, it would seem that resistance may be used as a measure of the effectiveness of copper sulphate as a fungicide.

When mercuric chloride was the killing agent, the results were much more complicated. The three low concentrations (Fig. 3) indicate again that the high concentration kills the organism more rapidly than the lower ones. As the concentration is increased (Fig. 9), however, other processes come into play. The very striking decrease in resistance, followed by a similarly striking increase where the strong solutions were used, is difficult to explain. However, if only the first part of the curves for all the concentrations of mercuric chloride are taken into consideration—the first ten minutes, for instance—the relative effectiveness of the various concentrations in decreasing the resistance is evident. The experiments on growth indicated that the cell is probably damaged beyond repair before the subsequent rise in the strong concentrations begins. On this basis, resistance may be used as a measure of the effectiveness of mercuric chloride in killing the organism as well as for copper sulphate.

The results with formaldehyde (Fig. 8) differ so radically from those obtained with other substances that they must be interpreted on a different basis. The curves all show an initial rise, followed by a gradual drop, which eventually falls to a point somewhere below the normal line, but generally not so far below as in the other cases. Osterhout (4, page 107) has shown that, at least in the case of *Laminaria*, the organism is injured when the resistance is above the normal as well as when it is below. In all probability the same is true with this fungus when it is treated with formaldehyde. It seems probable that an increase and a decrease in resistance both may be used as a basis for determining injury. Some experiments with strong formaldehyde tended to confirm this theory. Further experiments in which different concentrations of formaldehyde are compared, and experiments on recovery similar to those which have been done on *Laminaria*, should be carried out to test this point.

The results obtained for these three toxic agents with *Aspergillus* indicate rather definitely, therefore, that, with this particular fungus at least, conductivity may be used as a measure of the relative effectiveness of fungicides. It would be extremely interesting to find out whether other fungi would behave similarly and whether other toxic substances would give similar types of curves. If these things were true, this would be a very valuable method of determining, rapidly and perfectly definitely, the relative toxicity of fungicides.

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BIOLOGICAL AND CULTURAL STUDIES OF EXOASCUS MIRABILIS

A. J. MIX

WITH TWO FIGURES IN THE TEXT

In a recent paper (2) an account was given of some studies on the biology of *Exoascus deformans* (Berk.) Fuckel and on the behavior of that fungus in pure culture. The present report is concerned with similar studies of *Exoascus mirabilis* Atkinson. As the methods employed were the same as those used with *Exoascus deformans*, a detailed discussion of them will be omitted, and a brief account will be given of the principal results obtained.

THE FUNGUS AND ITS EFFECT ON THE HOST

Exoascus mirabilis is common in eastern Kansas, causing deformation of the shoots of the Chicasa plum, *Prunus angustifolia* Marshall. The characters of the fungus and the symptoms of the disease produced are well described by Atkinson (1) and the description will not be repeated. A deformed shoot is shown in figure 1.

The disease makes its appearance in Kansas late in April, usually one or two weeks later than peach leaf curl. Mature ascospores may be found the last of May or the first of June, these appearing a little later than the ascospores of *Exoascus deformans.*

Twigs and leaves of the current season's growth are affected, the hypertrophy never extending back into the wood of the previous year. Terminal shoots are more frequently affected than laterals. This may be explained, at least in part, by the habit of growth of the tree, since lateral buds usually produce short spurs, and seldom develop into rapidly elongating shoots. Young trees, one to three feet high, are more severely infested than older trees. The reason for this is not apparent.

Soon after the formation of ascospores the affected part of the twig is overrun with saprophytic fungi, and a little later it shrivels and dies. These dead and dried-out twigs may be observed on the tree for a year or more. The amount of injury to the tree is not great, and, since the host has no economic importance, the disease cannot be said to have any.

No affected fruits of *Prunus angustifolia* have been found, although Atkinson (1) describes deformation of fruits of this species by *Exoascus mirabilis* var. *tortilis.*

ISOLATION OF THE FUNGUS

Isolations were made from ascospore-bearing material in 1922 and again in 1924. Isolations from the interior of diseased twigs were made in 1922 and 1923. The number of successful isolations of the latter type was thirty out of a possible ninety-six, indicating that the fungus is somewhat more readily isolated from the interior of diseased tissues than is *Exoascus deformans.* The total number of cultures under observation during these studies was fifty-nine.

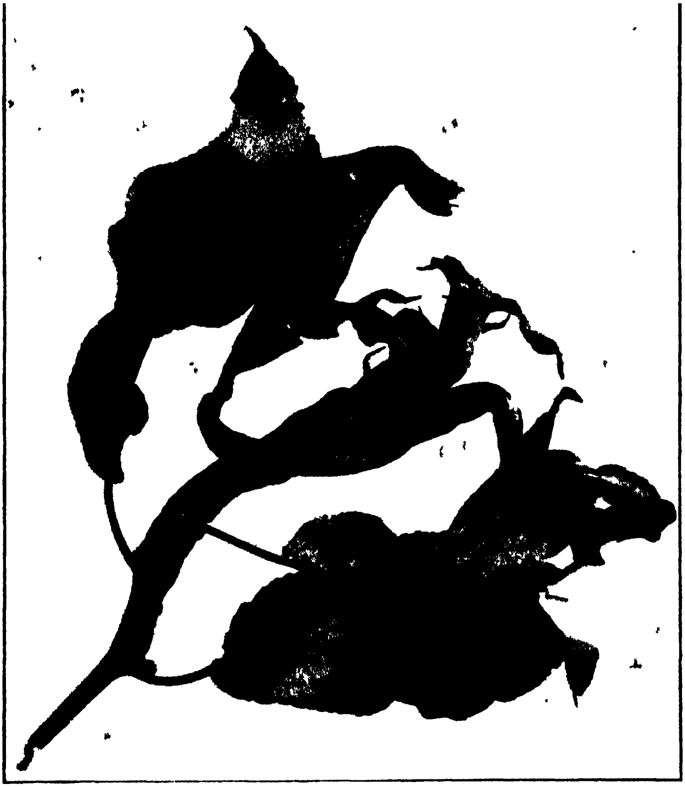

FIG. 1. A Chicasa plum shoot deformed by *Exoascus mirabilis.* Infection occurred early in the season.

BEHAVIOR OF THE FUNGUS IN CULTURE

The fungus closely resembles *Exoascus deformans* in habit and character of growth in culture media, the only distinction between the two being a very slight difference in color of the colony. When both organisms are grown on the same media the colonies of *Exoascus mirabilis* show a little more pink than those of *Exoascus deformans.* This color difference cannot be definitely expressed: colonies of both organisms on potato dextrose agar approximate 9″ f, pale vinaceous pink, of Ridgway's color chart, but their color really lies between that and white.

Favorable media for the growth of *Exoascus mirabilis* are: potato dextrose agar, potato dextrose gelatin, potato dextrose broth, sweet potato broth; sweet potato, carrot, and beet plugs; bean pods. Sweet potato plugs seem to favor the formation of the so-called resting cells of the fungus, and bean pods are favorable to the formation of actively budding cells, germ tubes, and mycelia. Media supporting less growth than those named are: potato maltose agar, string bean agar, and steamed rice. Potato sucrose agar gives very restricted growth. Plugs made from old potatoes, which may be believed to contain appreciable amounts of sugar, have been found to support nearly as much growth as other vegetable plugs. On plugs prepared from new potatoes almost no growth occurs. The fungus refuses to grow on beef agar, beef gelatin, and in beef broth. No synthetic media have been tried.

MORPHOLOGY IN CULTURE

Cells formed in culture are for the most part oval "conidia" which multiply by budding. In size these conidia range from 2.4 x 4.0 to 5.2 x 8.0 microns. In some cultures, especially in those on bean pods, there occur conidia which are oblong in shape. When the culture is not actively growing, budding is interrupted and the conidia then appear as oval cells with vacuolate protoplasm. So-called resting cells are also formed. These are round, oval, or oblong, and possess thicker walls than the conidia. In size they range between 4.0 x 4.8 and 12.0 x 17.8 microns. They germinate by rupture of the wall and the emergence of a thin-walled cell, and, as was suggested in connection with *Exoascus deformans*, it seems possible that they may represent ascogenous cells.

Germ tubes and short mycelia are also found in culture. These latter are occasionally several cells long and often quite irregular in diameter.

Finally, it should be stated that there are no observable microscopic characters by means of which cells from cultures of *Exoascus mirabilis* can be distinguished with certainty from those of *Exoascus deformans*.

TEMPERATURE RELATIONS IN CULTURE

Some study was made of the effect of temperature on the rate of growth of this organism in culture. Incubators adjusted to cover the entire range of growth were not available. It was possible, however, to learn something of the more important temperature relations. In table 1 are given data on growth of *Exoascus mirabilis* on plates of potato dextrose agar subjected to different temperatures. Rate of growth was determined by measuring the diameter of the colonies. No attempt was made to measure colonies more closely than to one-half millimeter. The values given in the table are averages of measurements of twenty colonies representing ten separate cultures of the organism.

TABLE 1.—*Growth of Exoascus mirabilis on potato dextrose agar at different temperatures*

Temperature	4–8° C.	8–12° C.	17–21° C.	25° C.	30° C.	35° C.
First test.						
Diameter of colony in millimeters.						
Ten days	3.4	3.8	5.0	4.6	1.4	
Second test.						
Diameter of colony in millimeters.						
Five days		3.3	6.0	4.5	2.2	0
Third test.						
Diameter of colony in millimeters.						
Five days					1.3	0

From an examination of this table it is evident that the minimum temperature for growth is below 8° C., the optimum in the neighborhood of 20° C., the maximum slightly above 30° C. These temperature relations agree with those previously found for *Exoascus deformans,* except that no growth of that organism occurred at 30° C. In the third test reported in table 1, cultures of *Exoascus deformans* were placed at temperatures of 30° C. and 35° C. along with those of *Exoascus mirabilis.* The cultures of *Exoascus deformans* failed to grow. This confirms the results already reported for that fungus (2).

DEVITALIZATION OF CULTURES BY HIGH TEMPERATURES

Six vigorous agar cultures of the organism were placed in incubators at 30° and 35° C. Daily transfers from these cultures to fresh tubes of potato dextrose agar showed that one culture was killed by six days exposure to 30° C., two more in seven days, one more in nine days, and the remaining two in ten days. This indicates that, although some growth may occur at 30° C., prolonged exposure to that temperature is fatal, and the critical temperature for the organism must be near 30° C. All cultures were killed by twenty-four hours' exposure to 35° C. It was previously found (2) that exposure for eight days or less to a temperature of 30° C. was sufficient to kill cultures of *Exoascus deformans.*

RESISTANCE TO DESICCATION

A test was made to determine the length of time that cells from culture would resist desiccation on glass. The details of the method have been previously described (2). It was found that the organism remained viable as long as 300 days when the glass slips bearing the film of dried cells were kept in the refrigerator at approximately 10° C.; that it survived similar exposure at room temperatures (20–25° C.) for 210 days, and at 30° C. for 160 days. This is evidence of considerable resistance to desiccation on the part of conidia of *Exoascus mirabilis,* and it is also apparent that desiccated conidia withstand a high temperature (30° C.) much better than conidia actively growing in culture media.

ACID-ALKALI RELATIONS IN CULTURE

The fungus was grown in plates of potato dextrose agar to which different amounts of five per cent or fifty per cent lactic acid, or of N/10 or N/1 NaOH had been added. Growth occurred on agar at pH 2.6, at pH 11.3, and at all intermediate concentrations tried. No growth occurred at pH 2.5 or at pH 11.6. Maximum growth occurred at pH 4.0 to pH 4.2.

The fungus was also grown in flasks of potato dextrose broth in which the hydrogen-ion concentration was varied by the addition of lactic acid or of sodium hydroxide in the same fashion as with the agar. Growth occurred in solutions whose initial hydrogen-ion concentrations were pH 2.81 and pH 10.45 respectively, and at all intermediate concentrations tried. No growth occurred at pH 2.69 or at pH 10.62.

Changes in the reaction of the broth during the first twenty days of growth were in the direction of increased acidity, except that solutions with initial hydrogen-ion concentrations from pH 3.0 to pH 4.0 remained unchanged during this period.

The data from which the conclusions just stated were derived will be presented in a later paper which will report a more complete study of the hydrogen-ion relations of *Exoascus mirabilis* and of *Exoascus deformans.*

INOCULATIONS

No extensive inoculation experiments have so far been performed. In 1923 the dormant buds on several twigs of *Prunus angustifolia* were sprayed with formaldehyde solution, 1 to 200. A few days later, when the tips of the buds showed green, inoculum from agar cultures of *Exoascus mirabilis* was inserted into the tips of the buds. No diseased shoots developed from any of these buds. In 1924, soon after the buds had opened, the young tips of several shoots were smeared with inoculum from agar cultures. The weather was unfavorable for infection and remained so for several weeks. The few diseased shoots that were finally found were thought to be due to natural infection.

OVERWINTERING AND INFECTION

The possession or lack of an overwintering mycelium remains an open question for several members of the Exoascaceae. No search for overwintering mycelium of *Exoascus mirabilis* has been made for the reason that diseased shoots invariably die, and it has not seemed worth while to look for such mycelium in the older, apparently healthy portions of the branches. The diseased shoots of the current season occur with no relation to those of the previous year, which is not what might be expected if mycelium remained alive in the neighborhood of diseased shoots throughout the year.

The spraying experiment reported below has a bearing on this question. On March 9, 1923, six trees of *Prunus angustifolia* were sprayed thoroughly with lime-sulfur solution, 1 to 20. Only one diseased shoot developed on any of these trees during the season. Counts made on June 7 on three nearby trees of approximately the same size as the sprayed trees gave a total of forty-two diseased shoots. This indicates that infection must be due to some overwintering spore-form, and that if overwintering mycelium exists it is a negligible factor in perpetuating the disease. What this overwintering spore-form may be is of course unknown, but it is evident that the conidia derived from budding ascospores possess two faculties: resistance to desiccation and the ability to live saprophytically, which should enable them to survive long enough to cause infection.

The following observations have a bearing on the questions of overwintering and infection. In this part of Kansas the early spring of 1924 was unusually dry. Rain occurred during the night of March 27, and there were showers the following day. This rain was insufficient to constitute what we may assume from our meager knowledge of infection phenomena in the Exoascaceae to be a good infection period, that is, a period of several hours during which the twigs and buds remain wet. With the exception of light showers on April 25, there was no more rain until April 28. During that night and the succeeding day, there occurred a prolonged rain, sufficient to constitute a good infection period, as described above.

Two twigs of the wild plum deformed by *Exoascus mirabilis* were found on April 25, and during the next two weeks careful search of a large thicket brought the number of diseased twigs observed up to eight. In a normal season the disease would have been abundant at this time. Discounting the possibility of perennial mycelium in these twigs, the disease must have resulted from infections occurring on March 28, and its scarcity may be attributed to the inadequacy of that infection period.

Following the rain af April 29, the weather remained fair until the latter part of May when several rains occurred. During the first week in June, many diseased shoots of the wild plum were found. This outbreak of the disease was quite as severe as that which may be observed earlier in a normal season. It is worth noting that the time between the supposed infection period and the discovery of the disease is the same for each outbreak, approximately four weeks. It is probable that diseased specimens might have been found a few days earlier in each case.

That there were two definite outbreaks of the disease in 1924, corresponding to two possible infection periods, is confirmed by the following observations. On June 10 the greater number of the diseased shoots of the second outbreak were bearing ascospores. In contrast to this, the few diseased shoots which had been located earlier were past this stage and were being overrun and killed by saprophytic fungi.

There was a further significant difference between the diseased shoots observed early in the season and those found later. The former exhibited the usual form of the disease, normal elongation of the shoot being pre-

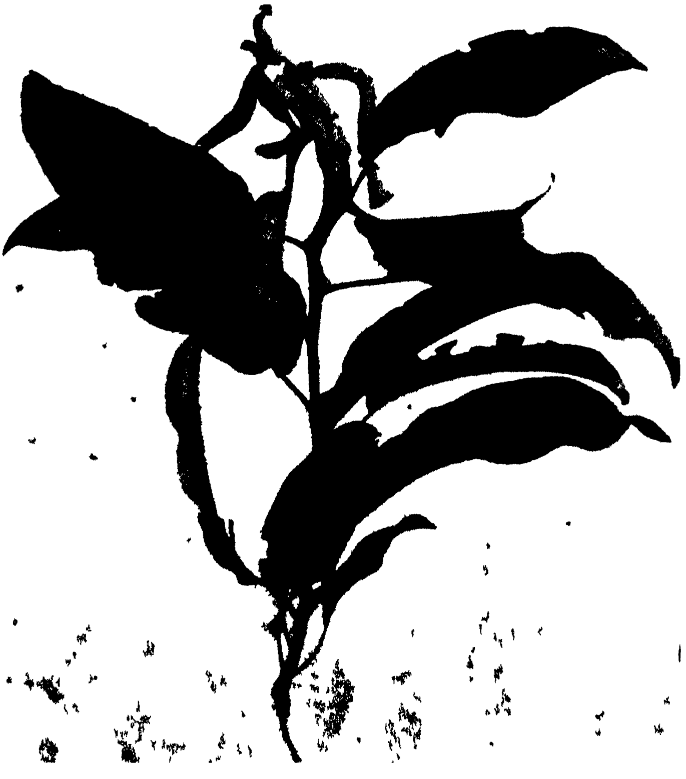

FIG. 2. A Chicasa plum shoot deformed by *Exoascus mirabilis*. Infection occurred after the shoot had made considerable growth.

vented and the deformation involving the whole shoot or all but a small basal portion. This appearance is shown in figure 1. It may be explained on the assumption that infection occurs soon after the unfolding of the bud. In marked contrast were the diseased shoots found in early June. Below the deformed terminal portions were several healthy internodes, as though infection of the growing tips had occurred after the shoots had made considerable growth in length. This appearance is shown in figure 2.

The observations reported above seem to warrant the following assumptions regarding the life-history of *Exoascus mirabilis*:

1. Overwintering mycelium, if such exists, must be a negligible factor in the incidence of the disease in the spring.
2. Infection must be due to some overwintering spore-form.
3. Infections may occur over a considerable period in the spring, depending on the frequency of infection periods.
4. Only the tender growing portion of a shoot is susceptible, the fungus being unable to deform the older, more woody part.

SUMMARY

A disease of the Chicasa plum, *Prunus angustifolia* Marshall, caused by *Exoascus mirabilis* Atkinson, is common in eastern Kansas.

The fungus has been isolated from the ascospore-bearing surfaces and from the interior of diseased shoots.

Colonies formed in culture are not easily distinguishable from those of *Exoascus deformans.*

Cells formed in culture are: budding conidia, resting cells, and short mycelia.

The minimum temperature for growth in culture is below 8° C., the maximum slightly above 30° C., the optimum near 20° C.

Cultures are killed by an exposure of ten days' duration or less to a temperature of 30° C., and by an exposure of twenty-four hours to 35° C.

Cells from culture resist desiccation on glass at 10° C. for 300 days, at 20–25° C. for 210 days, at 30° C. for 160 days.

Growth limits on potato dextrose agar are pH 2.6 and pH 11.3. Maximum growth occurs at pH 4.0 to pH 4.2.

In potato dextrose broth, growth occurs between pH 2.81 and pH 10.45. Growth of the organism induces increased acidity in potato dextrose broth cultures during the first twenty days, except that cultures between pH 3.0 and pH 4.0 remain unchanged.

Attempts at artificial inoculation have so far failed.

The disease may be controlled by dormant spraying. From this fact, and from observations on infection and progress of the disease, it is deduced

that overwintering mycelium is lacking or unimportant, that infection is due to overwintering spores (perhaps conidia), that infections may occur over a considerable period in the spring, and that only the growing tips of shoots are susceptible.

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BLOSSOM-END ROT OF PEPPER (CAPSICUM ANNUUM L.)

B. B. HIGGINS[1]

WITH FIVE FIGURES IN THE TEXT

Like the tomato and some other fleshy fruits, the pepper is subject to a physiological rot or spotting of the blossom end of the fruit, which, so far as I can learn, has previously been mentioned only once (3).

The disease is first manifested by one or more slight depressions at or near the blossom end of the half-grown fruit (Fig. 1). The depressed spot is usually slightly paler than the surrounding healthy tissue, but sometimes becomes darker green with a water-soaked appearance. The spots enlarge to some extent, but, unlike the tomato blossom-end rot spots, never involve the entire blossom end of the fruit, unless they become infected and enlarged by microorganisms.

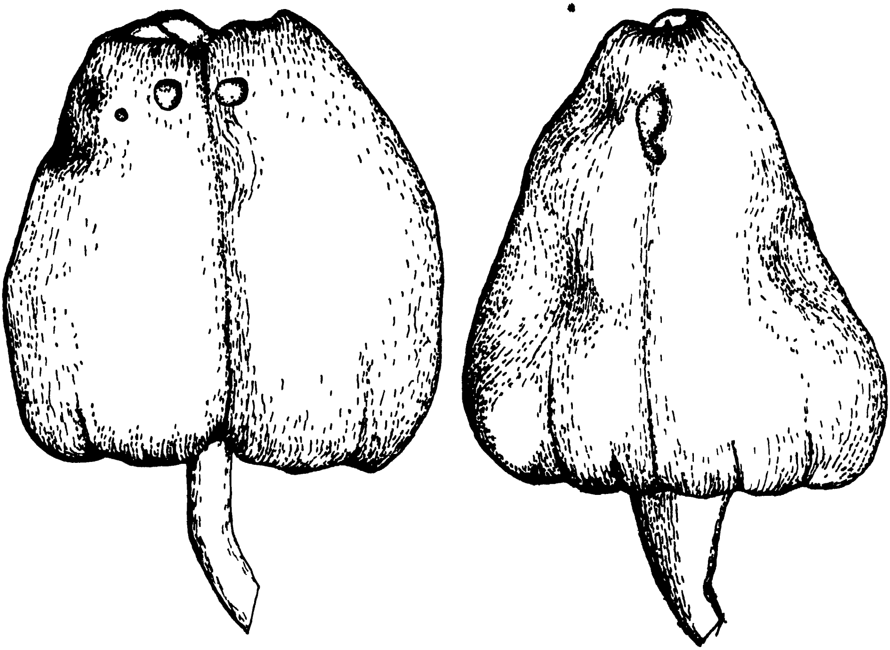

FIG. 1. A, Sketch of Pimiento pepper fruit showing single blossom-end rot spot fourth day after disease became visible (natural size). B, Sketch of Bell pepper fruit showing location and size of blossom-end rot spots second day after disease became visible.

[1] Paper number 18, Journal series, Georgia Agr. Exp. Sta.

In the field, infection by fungi and bacteria usually occurs, and after a few days the spot may have all the appearance of having been produced by the invading organism. In Georgia the most common and conspicuous invader of the spots is a species of Alternaria; and, from reference in plant disease literature, one may infer that this is true in other regions. In some cases as high as 45 per cent loss has been attributed to an Alternaria or Macrosporium rot; but, apparently no one has ever succeeded in producing a similar rot by inoculating with the fungus reported as the causal organism.

While studying this so-called Alternaria rot in 1922, the true nature of the origin of the spots was first suspected. During that year heavy rains occurred throughout May and early June. Pepper plants, after being set in the fields, grew rapidly and, by the end of the rainy period, many had set one to five fruits. During the latter part of June and throughout July very little rain fell. No fruit rots had been found during the rainy period. I was therefore much surprised to find during the succeeding very dry weather a large percentage of the fruits decaying at the blossom end. In one field approximately 90 per cent of the early fruits became spotted.

A large number of these spots were removed aseptically and the interior diseased tissue plated out on various media. Some platings yielded cultures of various organisms, but cultures from very young spots remained sterile.

With the exception of one species of *Alternaria* (probably *Alternaria solani*), none of the organisms isolated were found capable of infecting sound pepper fruits. Under very favorable conditions this organism was able to infect sound young fruits, but only very small spots, one to two millimeters in diameter, were produced. These spots in no way resembled the spots occurring in the field.

From these observations and from comparison with analogous diseases of tomato and other fleshy fruits, it was concluded that the disease was of purely physiological origin and was closely associated with the water relations of the plant.

The conclusion was further strengthened by field observations in 1924. In a one-acre field on the Station farm, pepper plants were set rather late in the spring. Their growth was further retarded by dry weather, and the first fruits were not set until the latter part of June. About this time a rainy period set in and lasted until the middle of July. During this period some rain fell almost daily, and both plants and fruits developed rapidly. On July 22 the entire field was examined carefully and not a single defective fruit was found. The earliest fruits were well matured at this time and subsequently ripened normally, but the younger fruits nearly all developed blossom-end spots during the ensuing drought.

EXPERIMENTAL RESULTS

In early summer of 1923, four plats, each six feet square, were set with 12 Pimiento pepper plants per plat. All were well watered and kept growing rapidly until August 20, when the first fruits were about an inch long. Water was then withheld from two of the plats. On August 30 two pods in these plats had developed spots at the blossom end. Between this date and September 24, ten more fruits in the dry plats developed spots, while none in the watered plats showed disease. Four of these spots were killed for histological study. The other eight were removed aseptically and placed on agar plates. All remained sterile.

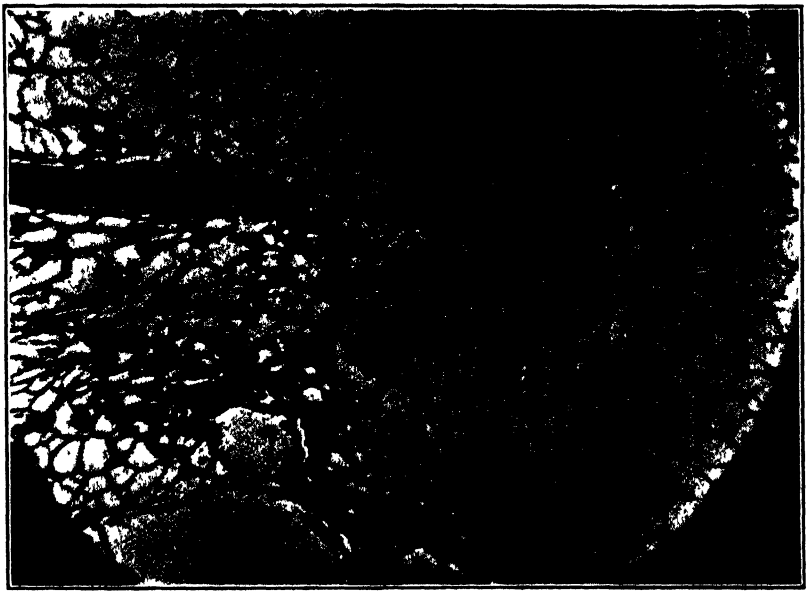

FIG. 2. Section of blossom-end rot spot, showing collapsed cells about end of vascular strand. Photomicrograph. × 65.

During the period of the experiment considerable rain fell. The air was moist and moisture seeped into the beds from the outside. It was therefore decided to run a series in pots so that the moisture could be better controlled.

On June 1, 1924, 26 plants of Pimiento pepper were set in 8-inch pots, and a similar number of Bell pepper plants were set in 12-inch pots. All were set on a greenhouse bench and were given plenty of water until fruits were about an inch long. Both the Pimiento and Bell series were then divided into two lots. Ten plants of each series were watered daily. The other 16 plants of each series received water at irregular intervals. Some were watered only when wilting was severe, and others received a small amount of water each evening. The latter system proved slightly better than the former, since it kept the plants fairly vigorous but allowed slight wilting in the middle of every hot, dry day.

During extremely hot, dry days of August, plants of the well-watered pots showed slight wilting, and a few fruits developed blossom-end spots. This might be expected of plants with such a limited root system as potted plants in saturated soil are likely to develop. On the Bell plants receiving limited water supply, 31 fruits, or approximately 25 per cent, developed blossom-end spots; while only 3, or about 2 per cent, of the fruits on well-watered plants were affected. Pimientoes affected were approximately 10 and 3 per cent, respectively.

HISTOLOGY OF THE SPOTS

The spots for histological study were removed and killed within less than six hours after they became visible on the surface. Most satisfactory results were obtained with material killed in Schaffner's chrom-acetic acid, or in a mixture of equal parts of 95 per cent alcohol and glacial acetic acid, and

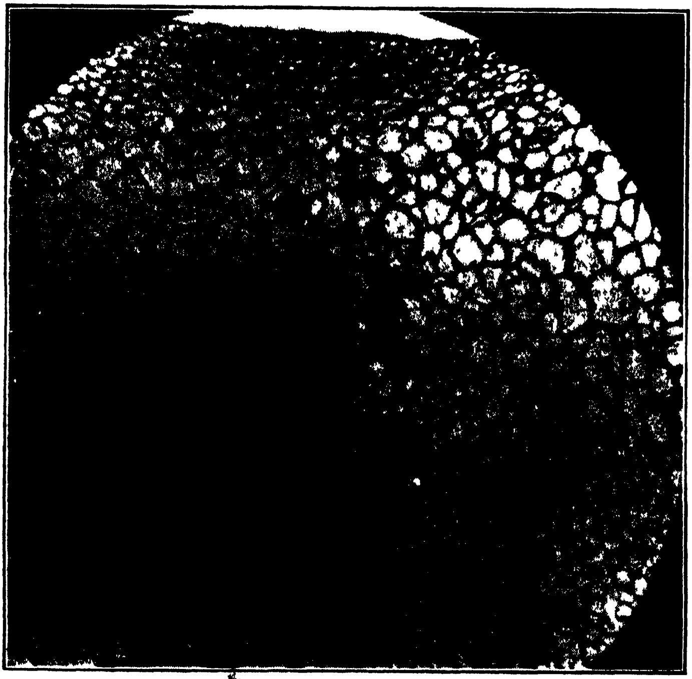

FIG. 3. Cross-section of spot, early stage, showing collapsed large cells abcve vascular strand. Photomicrograph. x 45.

stained with Haidenhain's haematoxylin. The flesh of the half-grown Pimiento pepper fruit is composed of a very thin cuticle, five to eight compact layers of comparatively small cells, about an equal number of layers of larger cells in which the small veinlets are embedded, and the single layer of very large cells lining the seed cavity. In the blossom end of the fruit there are numerous veinlet endings.

All the cells appear to be formed in the very young fruit, and the later enlargement of the fruit is due to the expansion of these cells. By the time the fruits are one and one-half to two inches long, certain cells lining the seed cavity have expanded until visible to the unaided eye as bladder-like projections over the inner surface, often a millimeter or more in diameter. The veinlets are surrounded by large, thin-walled cells.

As the fruit matures, the cell walls of the outer five to eight layers become cutinized; but this cutinization does not begin until the fruit is nearly full size, in vigorous plants when two to two and one-half inches in length

It is near the end of the stage of rapid cell expansion, but previous to heavy cutinization, that the blossom-end spots are formed The protoplasmic layer in the large cells surrounding the veinlet endings is very thin, and doubtless has very little power of recovery when once plasmolized. In the formation of the spots, these are the first to collapse (Figs. 2 and 3). Very often the tissue splits in this region and continues to collapse on the outer and inner surface layers (Fig. 4). In other cases collapse of the inner tissue is rapid and it does not split off from the outer layers.

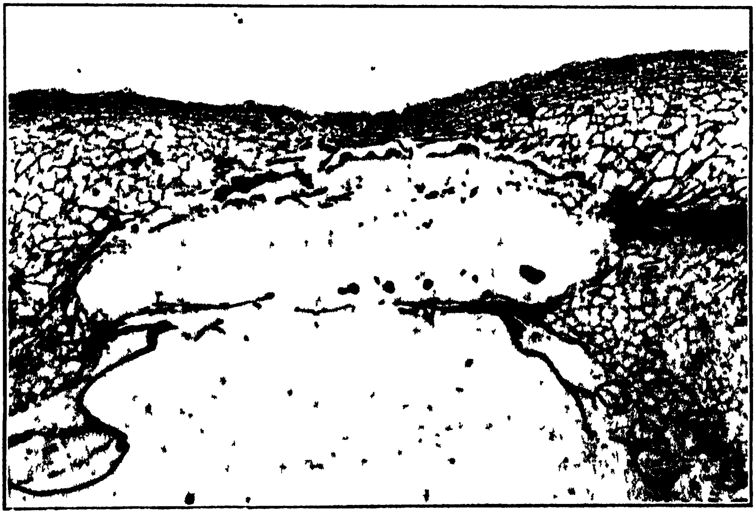

FIG. 4. Section of older spot, showing total collapse of inner tissue. Photomicrograph. ×25.

DISCUSSION

Field observations and experimental evidence have shown that two conditions are necessary for the production of blossom-end rot: (1) rapid succulent growth of the pepper fruits; (2) drought conditions must follow at once before the fruits become heavily cutinized.

The rot or spotting is always most severe on young plants. Under our field conditions a large percentage of the first-formed fruits are damaged, while those set later in the season are not severely damaged, unless there are extreme variations of wet and dry weather.

There are at least two reasons for the greater frequency of spotting of fruits on young plants. First, only a small number of fruits (one to five) are on the plant at this time and they grow larger and more rapidly than those on older plants which may support from fifteen to twenty-five growing fruits. The other is the poorly developed, shallow root system of the young plants which is incapable of obtaining sufficient moisture when the surface soil becomes dry, while older plants may be able to sustain growth from subsoil moisture.

Brooks (1) noted that blossom-end rot occurred on tomato plants growing in a nearly saturated soil, and from this he drew the conclusion that some factor other than drought might cause the rot. It may also be noted that on very hot, dry days spots developed on a few fruits on potted plants in wet soil; but I think the explanation lies in the poor development of young roots in a nearly saturated soil.

The vascular system of a pepper plant is more than sufficient to conduct an abundance of water to the leaves and fruits. Stems have been cut away until they were too weak to support their own weight, yet the remaining vascular tissue supplied sufficient water to prevent wilting of leaves or spotting of fruits. The roots are the limiting organs for water supply.

According to my observations on peppers, blossom-end spots never develop unless there is a slight drooping or wilting of the plant for at least a part of the day, and all cases of spotting may be explained by consideration of water relations.

The exact explanation of spot formation is not a simple matter however, because water relations between various organs of a plant are so imperfectly understood. Brooks (1) assumed that, in case of tomato blossom-end rot, water was actually withdrawn from the fruits, whenever a shortage of water occurred in the plant, by means of the greater concentration of solutes in the leaves. A similar assumption was made by Mix (4) in explaining the development of "drouth spot" in apples. Chandler (2) has shown by experimental evidence that such withdrawal of water from fleshy fruits for the support of leaves does occur in a large number of plants; and this has been confirmed for pepper by my own experiments. Leafy branches removed from the plant wilt and dry more rapidly when the fruits are removed than when the fruits remain attached to the branch, and fruits attached to a severed leafy branch wilt more rapidly than those removed from the branch. It seems, therefore, that this sudden withdrawal of water by the wilting leaves is the primary cause of the spots. The negative pressure developed in the vascular system of the fruit causes withdrawal of water first from the cells surrounding the veinlet endings. These cells, poor in solutes and in protoplasmic content, have very little power of recovery after plasmolysis. This is especially true for young fruits in which the cells have reached nearly their maximum expansion but as yet contain little stored reserve food. The heavy cutinization of the outer cell layers of older fruits will usually prevent the collapse of this tissue, even when the interior cells become plasmolized.

SUMMARY AND CONCLUSIONS

1. A physiological spotting of the blossom end of pepper fruits is very common in the field and is the indirect cause of heavy loss to pepper growers.
2. The disease develops almost exclusively on half-grown, green fruits.
3. It is caused by drought following a period of rapid growth of the pepper plants and fruits.
4. The disease has been produced experimentally by withholding water from plants when the fruits were half-grown.
5. The spots are initiated by the collapse of the large, thin-walled cells surrounding the ends of vascular strands.
6. The spots usually become infected and enlarged by microorganisms.
7. Because of the small size of the pepper plant and the ease with which it can be grown under controlled conditions, the small size of the spots, and the thinness of the tissues involved, it is admirably suited for the study of drought spots.

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A ROT OF APPLES CAUSED BY BOTRYOSPHAERIA RIBIS

E. Aline Fenner

Considerable literature has been published in the past regarding *Physalospora malorum* (Peck) Shear (*Sphaeropsis malorum* Peck) as a cause of black rot of the apple. Yet no one has made a systematic study of this disease of apples collected over a wide range of territory in the United States, nor has anyone done any intensive work in testing the pathogenicity of the organism causing it. The evidence given in this paper endeavors to establish the fact that in certain parts of the United States another fungus quite different from *P. malorum,* though related to it, is responsible for a rot very similar to the true black rot of the apple.

It was while engaged in some studies of black rot, in association with Dr. J. S. Cooley, the results of which will be published shortly, that this new rot was found. The causal fungus of this rot is *Botryosphaeria ribis* G. and D. (2). Morphological and cultural studies as well as inoculations have fully established the identity of the fungus from the apple rot tissue with that already known on apple and currant twigs. In view of the striking resemblance between the two fungi, not only as rots on apples, but also in culture, it is quite possible that some investigators have unwittingly been dealing with *Botryosphaeria ribis* instead of *Physalospora.*

In pursuing the investigations just referred to, apples were collected in 1923 from Mt. Alto, Pa.; Rockville, Md.; Middletown and Roanoke, Va.; Cornelia, Ga.; Bentonville, Ark.; Salem, Ind.; and Guelph, Ontario. Fungi were also isolated from rotting quinces and Kieffer pears. From all macroscopic appearances the fruit was infected with *Physalospora malorum,* some apples showing abundant young fruiting bodies and the concentric zones so characteristic of black rot. Even the sub-cultures of the fungi made from the decayed pulp, when grown on cornmeal agar, were so similar that no differences were noticed. It was only after the imperfect stages of the fungi fruited and a microscopic examination of the spores was made that the presence of the two distinct fungi was established.

The fungi were induced to fruit by following the methods employed by Shear, Stevens, and Wilcox (4). Transfers were made from the original sub-cultures to cornmeal paste in 100 cc. Erlenmeyer flasks. These flasks were kept in a greenhouse at Arlington Farm, Va., at a temperature ranging from 50°–70° F., with a much warmer temperature at midday. After a period of four to six weeks the cultures were carefully examined. Practically all of the ninety-seven cultures had fruited and a microscopic study revealed that ten of them, more than ten per cent of the total number, were quite different from the *Physalospora malorum* which was expected. The spores of these fungi were hyaline and narrower than those of the pycnidial form of *P. malorum,* measuring 15–26 × 5–6 µ with an average of 20 × 6 µ. Careful study and comparison convinced the writer that they were the Dothiorella stage of *Botryosphaeria ribis.* Three of the apples infected with *B. ribis* came from Cornelia, Ga., one from Bentonville, Ark., and four from Arlington Farm, Va. The other two were made from Kieffer pears from Arlington Farm. That this fungus actually was *Botryosphaeria ribis* was strikingly confirmed by the fact that one of the cultures proved to be the pathogenic *Botryosphaeria ribis chromogena,* the cause of currant cane blight.

This particular culture isolated from an apple collected at the U. S. Experiment Farm at Arlington, Va., showed the deep pink color characteristic of the currant cane blight organism, when grown on a very starchy medium (2). To determine whether this chromogenic fungus was identical with the currant cane blight fungus, some inoculation experiments were made by Dr. Neil E. Stevens at Toms River, N. J. In the spring of 1924 two dozen currant plants of the "Wilder" variety were secured from a nursery in northern New York and planted in a location well removed from other currants. Early in June, when the plants had reached a stage in their growth which seemed most favorable for infection (2), one or more canes on each of eighteen were inoculated with mycelium from a pure culture of the chromogenic form from the apple. In all, twenty-six apparently sound currant canes were inoculated, six plants being kept for controls. The methods used were the same as those employed by Stevens and Jenkins (5). A small incision was made in the bark with a flamed scalpel and mycelium of the fungus inserted in the wound. Moist cotton was then bound around the cut. The control plants were wounded and tied up in a similar manner but without the inoculum. On August 10, after a period of two months had elapsed, twenty-two of the inoculations, or more than three-fourths of those made, had taken effect. The appearance of the dead canes was identical with that of canes killed by the currant cane blight fungus, and from the pith and wood of many of them isolation cultures were subsequently made. These cultures were compared with those of *Botryosphaeria ribis* and, so far as the writer could observe, were identical with them. The control plants and all canes not inoculated remained in a healthy condition.

That these fungi do actually cause a rot of apples was proved by inoculations. In the winter of 1923 and 1924 Grimes Golden, Ben Davis, and Jonathan apples were inoculated with mycelium from the ten cultures of *B. ribis* along with those of *Physalospora malorum.* The fruit was carefully selected in order to obtain uniformity of size and ripeness, care being exercised to discard any apples bearing stem punctures or other abrasions. After a thorough washing and rinsing in fifty per cent alcohol followed by sterile water, the apples were placed in damp chambers containing wet paper to maintain the proper moisture conditions. Inoculations were made by puncturing the skin with a sterile needle and introducing some of the mycelium. The inoculated fruit was kept at room temperature about five or six days and at the end of this period the rots were inspected. The apples were cut transverse to the core along a plane running midway between the calyx and stem-end. As in the case of the original apples from which the isolations were first made, the *B. ribis* rot was not distinguishable from the black rot caused by *P. malorum,* neither in respect to the decayed fleshy tissues of the apple nor in the general appearance of the skin. Even the measurements of the two rots varied through much the same range.[1] In fact, many efforts were made to find some conspicuous differences between the two rots on apples whereby one might distinguish the one from the other. Slight differences were observed but the rots caused by each fungus, however, showed such variations in respect to taste, firmness and appearance that repeated examinations of diseased fruits failed to reveal any definite means of discrimination.

In the autumn of 1924, fifty-eight more isolations were made from apples collected in Durham, N. H.; Snow Hill, Md.; Winchester and Covesville, Va.; Ft. Valley, Ga.; Bentonville, Ark., and Salem, Ind. In the study of these cultures the same method of procedure was followed that was used in the preceding year. When cultured on cornmeal in flasks, all the fungi fruited and among the number three chromogenic forms were found, one isolated from an apple collected at Covesville, Va., another collected at Ft. Valley, Ga., and a third at Arlington Farm, Va. Of the remaining fifty-five cultures six proved to be nonchromogenic *Botryosphaeria ribis,* two coming from Arlington, three from Ft. Valley, Ga., and the sixth from Winchester, Va. In all, over fifteen per cent of the cultures isolated from spots which were apparently black rot, proved to be the currant cane blight.

One of the most interesting phases of the second year's work was the appearance of the purplish pink color on certain apples artificially infected with the chromogenic form from Arlington Farm. This condition was not observed in the inoculations the first year, probably because the apples used in 1924 were decidedly green compared to the ones inoculated in the autumn of 1923 and therefore more starch was present in the tissues of the fruits. The results, then, in the two successive years were as might be expected, since it has already been noted that chromogenesis is in evidence only when the fungus is cultured on a very starchy medium. The results of this season's work not only confirm the data from last year's experiments, but the discovery of chromogenic forms from Covesville, Va., and Ft. Valley, Ga., shows that the fungus is not confined to any one region. It is quite possible in fact that *Botryosphaeria ribis* is rather generally present as a rot of apples in many parts of the United States.

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[1] Graphs showing the measurements of the rots will appear in a joint publication with Dr. J. S. Cooley.

That *Botryosphaeria ribis* occurs on cut apple limbs was announced about one year ago by Shear, Stevens, and Wilcox (4), but this fungus has never been reported in the United States as a rot of apples. Putterill (3), however, described a fungus in 1919 which caused a canker of apple trees in South Africa and which he also showed by artificial infection would cause a rot of apples. Although closely resembling *B. ribis* even to the chromogenic condition associated with the currant parasite, Putterill claimed the fruiting bodies of this fungus varied in size from those of the currant cane blight organism, and for that reason gave it the name *Botryosphaeria mali.* Shear, Stevens, and Wilcox (4) attach little significance to this difference as the stromata of the currant cane blight fungus are variable, their size depending to a great extent on the thickness of the bark on which they grew. The discovery that *Botryosphaeria ribis* causes a rot on apples in the United States strengthens the opinion already advanced (2) that Putterill's fungus and the one discussed here are one and the same organism.

Birmingham (1) recently reported a canker of apple trees in New South Wales caused by a fungus which he called *Dothiorella mali,* E. and E. His description of the fungus agrees with *Botryosphaeria ribis.* The writer has just received pure cultures of the apple fungus from Birmingham and has found that it develops on cornmeal flasks the chromogenesis characteristic of *Botryosphaeria ribis chromogena,* the currant cane blight fungus.

Birmingham does not mention the presence of this organism on the fruit of the apple but merely on the wood. However, the writer has recently inoculated apples with pure cultures of the fungus sent by Birmingham and has found that it causes a rot apparently identical with that caused by *Botryosphaeria ribis.*

SUMMARY

Evidence is presented in this paper to show that a fungus which causes a rot on the apple is identical with *Botryosphaeria ribis* and its form *B. ribis chromogena.*

The rot is very similar to that caused by *Physalospora malorum* and may easily be mistaken for true black rot.

When currant canes are inoculated with mycelium from the chromogenic form isolated from apples collected from widely separated regions, a disease

is produced apparently identical with that caused by *Botryosphaeria ribis chromogena.*

The identity of the fungus has been established by its morphological character, including spore measurements, and its pathogenic nature proved by several inoculation experiments both on apples and currant canes.

Apparently this fungus is identical with that reported by Putterill on apples in South Africa under the name of *Botryosphaeria mali,* and with that reported in New South Wales by Birmingham as *Dothiorella mali.*

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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EFFICIENCY OF A SELF-MIXING DUSTER

RALPH E. SMITH

WITH ONE FIGURE IN THE TEXT

One of the obstacles to the success of the dusting method of applying fungicides and insecticides is the fact that mixtures containing different materials or proportions must be made up in the factory or by a separate preliminary mixing operation and cannot be varied or changed at will during the process of application. Furthermore, the extra cost of mixing, packing and transporting a large amount of low-grade filler like lime or sulphur adds a disproportionate amount to the price of the finished material by the time it reaches the farm. In the case of nicotine dust, a useful addition to many fungicidal materials, these considerations are particularly important. Nicotine is high priced and volatile and if the farmer could buy his own materials and mix the dust when and as needed, considerable economy could be effected together with the advantage of a freshly made mixture.

In a recent publication[1] we suggested the idea of a "self-mixing" duster —that is, a dusting machine having an agitator in the hopper so that dry pulverized materials like hydrated lime, sulphur, de-hydrated copper sulphate and commercial Bordeaux powder could be mixed together in any desired proportion in the machine itself. Nicotine dust is commonly prepared by mixing one of the commercial liquid preparations containing 40% nicotine as nicotine sulphate, with hydrated lime or sulphur, or a mixture of the two. In the proportions commonly employed the excess of dry material is sufficient to absorb the necessary amount of liquid and still remain in a dusty condition.

Objection has been made to the self-mixing idea as applied to nicotine dust on the ground that a sufficiently thorough mixture could not be made in this way. This depends, naturally, on the efficiency of the particular type of machine employed. That self-mixing can be applied in making nicotine dust was demonstrated by the following test, using a machine built in cooperation with this Division by the Bean Spray Pump Company of San Jose, California. Forty pounds of hydrated lime was dumped into the hopper of the duster and a two-pound can of "Hall's Nicotine Sulphate" (40% nicotine) sprinkled over it. The lid was then closed and the machine allowed to run for two minutes. At the end of this time a pint

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[1] Smith, Ralph E., and J. P. Martin. A self-mixing dusting machine for applying dry insecticides and fungicides. Cal. Agr. Exp. Sta. Bul. 357: 497–506. 1923.

Dusting walnut trees with a self mixing machine Dust contains hydrated lime, dehydrated copper sulphate and nicotine

glass fruit jar was filled with dust from the top of the mass (sample No 1) and another from near the bottom (No. 2). The hopper was then closed and the machine run eight minutes longer. At the end of this time two more samples were taken (Nos 3 and 4). The four jars were sealed and forwarded to the Division of Chemistry, California State Department of Agriculture, Sacramento, for determination of nicotine The result was reported as follows: Sample No. 1, nicotine 1 89%; No. 2, 1 89%; No. 3, 1.88%, and No. 4, 1.87%. Theoretically, the mixture contained 5% of the 40% nicotine solution, or 2% actual nicotine. A small amount of the solution probably stuck to the can in pouring into the hopper, but uniformity of mixing was the main consideration in this test rather than the actual amount of nicotine. The high degree of uniformity obtained throughout the whole mass of dust, even after the two-minute period, will be noted.

It is sometimes necessary to use a dust mixture containing considerably more nicotine than this. Another test was therefore made by using five and one-half pounds "Black Leaf 40" (40% nicotine, as sulphate) to 50 pounds of dust. This is 11% of the commercial solution, or 4.4% actual nicotine. A pint sample of the resulting mixture was sent to Sacramento for testing. The report showed the actual amount of nicotine in the sample to be exactly the same as the theoretical—namely, 4.4%. This represents about the limit of the use of the 40% solution for making nicotine dust, as the mixture tends to be moist and sticky.

These samples were taken from the hopper before the material had passed out into the discharge pipe. During the latter operation, in the type of machine developed by us, the dust passes through the fan. This breaks up any soft lumps and carries the mixing process still further. The demonstrated efficiency of machines representing the self-mixing idea and the great advantages, financial and otherwise, of such a device make the wider knowledge and application of this principle highly desirable. The cost of the dusting can be reduced and greater efficiency obtained by this method, particularly in the case of mixtures containing nicotine. The advantage of being able to vary the mixture at will at the time of application, just as different materials or amounts can be put into the tank of a liquid sprayer, is also an important one.

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A NEW FOOT ROT OF THE SWEET POTATO

J. J. TAUBENHAUS

WITH ONE FIGURE IN THE TEXT

This disease was first met with by the writer in 1914 and 1915 while he was connected with the Delaware Agricultural Experiment Station. The trouble was found at Seaford, Delaware, on tomato seedlings and sweet potato sprouts in cold frames. Cultures then made yielded a species of Macrosporium. However, owing to pressure of other work and change of positions, the writer was unable at that time to carry on further studies of this organism to determine its relationship to the foot-rot disease. During the last few years (1919 to 1921), while studying sweet potato and tomato disease at Troup and Jacksonville, Texas, a foot rot was found on sweet potato sprouts. The same trouble was also found on tomato seedlings in cold beds. In this connection it should be added that during 1920 Rosenbaum[1] described a foot rot of tomatoes from Delaware and New Jersey, which he attributed to the fungus, *Macrosporium solani* E. and M. In 1921, Pritchard and Porte[2] described a collar rot of tomato plants which they thought to be the same or similar to the one described by Rosenbaum, but which they believed to be caused by the fungus, *Verticillium lycopersici* Prit. and Port., although they also added that under certain conditions the same disease may be induced by *Macrosporium solani.*

The symptoms of foot rot both on sweet potato and tomato plants in Texas are characterized by deep, dark lesions at the foot of the plant (Fig. 1), which resemble the injury from Rhizoctonia. When these lesions become numerous, the affected host topples over and breaks. Numerous isolation cultures of the Texas material, both from sweet potato and tomato plants, frequently yield a pure growth of a Macrosporium fungus. It therefore became necessary to determine whether this disease was similar to the one described by Rosenbaum. Unfortunately, we were unable to secure cultures of his Macrosporium. In order to carry out inoculation experiments, sixteen 12-inch pots were filled with good garden soil and steamed for six hours at 15 pounds pressure. After cooling down, eight pots were planted with healthy, six-week-old tomato plants, and the renaining pots with healthy sweet potato sprouts. Six pots of each were ised for inoculation purposes, and four as checks. The plants were permitted to grow undisturbed for several weeks In this way both tomato ind sweet potato plants made some new growth of stems and roots. The nethod of inoculation consisted in introducing into the soil an eight-day-old

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[1] Rosenbaum, Joseph. A stem disease of tomato caused by *Macrosporium solani* E. and M. (Abstract) Phytopath. 10: 59. 1920.

[2] Pritchard, F. J., and W. S. Porte. Collar rot of tomato. Jour. Agr. Res. 21: 179–184. 1921.

pure culture of strains of the Macrosporium as they were isolated from affected sweet potato and tomato plants and grown on potato agar slants. The organism from the slant was first broken up in sterilized water, then poured around the foot of the plant and covered with sterilized soil. The inoculated pots were kept moist by watering with sterilized water without, however, covering them with bell jars. The check plants were untouched, but were separated from the inoculated ones to avoid accidental infection. The results of these inoculations are given in table 1.

TABLE 1.—*Results of inoculating tomato and sweet potato plants with Macrosporium*

Source of organism	Host inoculated	Date inoculated	No. of pots inoculated	Result and date
Pure culture Macrosporium isolated from foot rot of tomato.	Tomato plants	May 10, 1922.	3	June 28, 1922. Typical foot rot lesions on 60 per cent of plants.
Check. No inoculation.	Tomato plants	Check. No inoculation.	2	June 28, 1922. All healthy.
Pure culture Macrosporium isolated from foot rot of tomato.	Sweet potato plants	May 10, 1922.	3	June 28, 1922. Typical foot rot lesions on 50 per cent of plants.
Pure culture of Macrosporium isolated from foot rot of sweet potato.	Sweet potato plants	do	3	June 28, 1922. Typical foot rot lesions on 50 per cent of plants.
Pure culture isolated from foot rot of sweet potato.	Tomato plants	do	3	June 28, 1922. Typical foot rot lesions on 30 per cent of plants.
Check. No inoculation.	Sweet potato plants	Check. No inoculation.	2	June 28, 1922. All healthy.

From this table it is seen that successful inoculations and typical foot-rot symptoms were obtained on the inoculated tomato and sweet potato plants when using a strain of Macrosporium fungus originally isolated from either of these two hosts. In this connection it should be added that reisolation cultures were made from the tissue of the artificially inoculated plants and the original fungus recovered.

Although no inoculations were made with the Macrosporium strain of Dr. Rosenbaum, it appears that the results shown in table 1 do not differ from those obtained by him. Further work is now in progress to determine whether the early blight of the Irish potato may be induced by using spores of *Macrosporium solani* from the foot rot of both sweet potato sprouts and tomato seedlings and vice versa.

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A FIELD TEST OF MERCURIC CHLORIDE SOLUTIONS IN POTATO SEED TREATMENT

L. J. CROSS

In the treatment of seed potatoes with corrosive sublimate solution, 1 to 1,000, for scab and Rhizoctonia, the strength of the sublimate solution decreases rapidly. To overcome this loss in concentration, some investigators advise the addition of one ounce of the sublimate to thirty gallons of the original solution after each treatment until it has been used five times, when it should be discarded. While this method tends to maintain the strength of the solution more nearly to the standard, yet the actual strength of the solution may be much higher or much lower than the standard, according to the condition of the potatoes used.

The withdrawal of the sublimate from the solution is not uniform with potatoes in different conditions. The more soil there is on the potatoes, the more rapidly will the sublimate be used up. The nature of the soil also has an influence upon the amount of sublimate withdrawn, some soils abstracting the sublimate more rapidly than others. Potatoes affected with decay or rot absorb the sublimate from solution very rapidly.

To overcome this objection, the following method of determining the strength of the sublimate solution has been devised. Solution required: Potassium iodide solution, five grams to the liter. Dissolve five grams of potassium iodide in distilled water and make to one liter.

Apparatus required:

1—25 cc. measuring cylinder.
1—100 cc. measuring cylinder.
1—1,000 cc. measuring flask
1—10 cc. pipette.
1—300–500 cc. beaker, low type.
1—liter bottle for the potassium iodide solution.

Procedure: Measure out 25 cc. of the potassium iodide solution and place in a beaker. Fill the 100 cc. measuring cylinder to the 100 cc. mark with the sublimate solution to be tested. Titrate by pouring small quantities at a time of the sublimate solution from the measuring cylinder into the beaker and shaking until a permanent pink color or cloudiness is obtained. Note the amount of sublimate solution used. A test made of the solution as made up before treatment of the potatoes is started is used as a standard on the concentration of the other solutions after use in treating potatoes.

If the solutions have been properly made, it should take approximately 50 cc. of the 1 to 1,000 (4 oz. to 30 gals.) mercuric chloride solution to give the end-point against 25 cc. of the potassium iodide solution. This standard may be determined by dissolving one gram of pure mercuric chloride in one liter of water and titrating 25 cc. of the potassium iodide solution with a portion as has been described.

The sublimate solution used in treating the potatoes soon becomes dirty, and in the test a decidedly milky appearance of the solution is the end-point of the titration. This end-point has been checked with the potassium cyanide method for the determination of mercury and gives concordant results.

After long use of the sublimate solution, it becomes more difficult to determine the end-point of the titration when 25 cc. of the potassium iodide solution is used. Then one may use instead 10 cc. of the potassium iodide solution and titrate from a 25 cc. cylinder of the sublimate solution. Titrate to a decidedly milky appearance. The standard then will call for approximately 20 cc. of the sublimate solution. The exact standard will, of course, be obtained by titrating with the known mercuric chloride solution mentioned heretofore. With these small quantities it is possible to test the sublimate solution in the treating vat after a 1,200 bushel run.

This test has been used for four years under the direction of the author and 90 to 100 per cent killing of Rhizoctonia has been obtained in the treatment of about 50,000 bushels of seed potatoes.

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PHYTOPATHOLOGICAL NOTES

A New Bacterial Disease of Alfalfa.—In September, 1924, Prof. L. F. Graber called the writer's attention to a field of alfalfa near Freeport, Ill., which had been dying in spots in an unaccountable manner during the summer. A small spot which appeared to winterkill in a six-year-old field had grown larger after each cutting by the failure of plants around it to recuperate, until it had become about an acre in area, extending into an adjoining year-old planting. New smaller spots of dying plants were appearing in both the older and younger portions of the field. Examination of the tap roots of those plants which were putting out weak dwarfed foliage after the second cutting showed a brown discoloration under the bark which in advanced cases would slip readily from the root, leaving a woody core, yellow on the outside, but commonly of the usual white color at the center. Microscopic examination of these discolored roots revealed that the color was associated with the presence of masses of bacteria in a large number of the outermost vessels of the wood. In young plants apparently recently infected only a few vessels were found filled with bacteria having little color in mass. Later the color of the bacterial mass was darker, and finally the vessels were filled with a yellow substance in which the individual bacteria were not seen. Vessels thus filled with bacteria were found not only throughout the length of the larger roots, but in some of the stems with reduced foliage as high as six inches above the ground.

A survey of the older alfalfa fields around Monroe, Wis., discovered four additional fields infested with this disease. In one case, seven acres of six-year-old Grimm had lost half of a fine stand during the summer, and very few living plants remained uninfected. Another similar stand of Grimm was hardly less completely destroyed. A few plants diseased in this manner were found in a field at Madison, Wis. It is a fact of possible significance that at least three of these diseased fields have grown alfalfa before the present stand was sown.

An attempt to isolate bacteria from plants in each of these fields has given cultures of what appears to be the same organism in each case, and inoculations have produced the disease in plants brought into the greenhouse. Meantime it is desired to learn the extent of this trouble, and specimens from suspected fields will be greatly appreciated. The discoloration of the wood of the tap root just under the bark appears to be a distinguished characteristic of this disease. Unless it shall appear later that unusual conditions have made possible an extraordinary spread of this trouble in the infested fields found, this disease appears to be a serious menace to alfalfa culture in the region where it has been found.—**FRED REUEL JONES**, Madison, Wis.

The weather and peach leaf curl in eastern Kansas in 1924.—The unusual weather of the spring of 1924 in the vicinity of Lawrence, Kansas, and its effect on a disease of the Chicasa plum caused by *Exoascus mirabilis* Atkinson has been described elsewhere (Mix, Biological and cultural studies of *Exoascus mirabilis.* Phytopath. **15**: 1925). The purpose of this note is to record the effect of the weather of that year and locality on the occurrence of peach leaf curl.

The following is a description of these abnormal weather conditions. There were showers on the twenty-seventh and twenty-eighth of March, without however developing into a rain of several hours' duration, such as is believed to be necessary for infection of peach leaves by *Exoascus deformans* (Berk.) Fuckel. A period of fair weather followed, broken only by showers on April 25, until a prolonged rain began on April 28.

The peach buds were beginning to open on March 28, and many infections might have been expected had the rain of that date been of longer duration. During April careful search was made in several unsprayed orchards in which peach leaf curl is usually abundant. On April 15 four curled leaves were found on one tree in one orchard. A short time later a few curled leaves were found on another tree in the same orchard. Except for these few specimens, no peach leaf curl could be found during that month. In a normal season the curl appears in this locality early in April.

On May 10 considerable curl was found. Most of this was on the one tree previously mentioned, but there was an occasional affected leaf on other trees. This late curl was different from the common type in that only small isolated areas of the leaf blade were involved. From one to several small lesions occurred on the same leaf. Apparently the leaf is more resistant to attack when partly grown, and the fungus is then unable to deform large areas of the blade.

The writer has observed this type of curl in other seasons and has believed it to be due to late infections, but evidence to prove this belief has been lacking. An experiment in artificial inoculation previously reported (Mix, Biological and cultural studies of *Exoascus deformans.* Phytopath. **14**: 217–233. 1924) shows that infection may occur after the leaves are expanded and partly grown.

The evidence here presented of two separate appearances of peach leaf curl, each following a possible infection period by about two weeks, seems to justify the following conclusions:

1. Infection of peach leaves by overwintering spores of *Exoascus deformans* may occur not only at the time of opening of the buds but also after the leaves are considerably expanded.

2. The peach leaf as it grows older is more capable of resisting the deforming effect of the fungus.

A further deduction from these observations is that overwintering mycelium of *Exoascus deformans*, if it exists at all, plays a negligible part in the incidence of the disease in the spring.—A. J. MIX, UNIVERSITY OF KANSAS, LAWRENCE, KANSAS.

An Easy Method of Mounting Superficial Fungi for Study.—In studying members of the Erysiphaceae, considerable difficulty has been found in quickly and easily obtaining mounts showing the vegetative parts of the fungus. When one desires to observe the relation of the conidiophores to the mycelium from which they arise and to study the catenulate nature of the conidia, it is necessary to have a slide showing the interwoven masses of mycelium in as normal a condition as possible. The method that has finally been evolved consists of using glycerine jelly slides which are easily prepared and used in the following way.

A camel's hair brush is dipped into melted glycerine jelly and a thin coat is spread over a part of a warm slide which is then allowed to cool. The leaf containing the mildew to be studied is pressed firmly with the fingers against the glycerine jelly slide. When the leaf is pulled away from the slide, much of the mycelium, conidiophores, conidia, and any perithecia present adheres to the surface of the glycerine jelly.

A mount made in this way may be observed under low power without a cover glass; or, by adding a few drops of water and then a cover glass, both low and high power may be used. Such a mount, after drying, is more than temporary, as it can be kept for some time; and its permanency may be increased by sealing with gold size.

This method is well adapted for teaching. The students are provided with glycerine jelly slides previously prepared. Their own mounts are made in the manner described with little difficulty. By this method the appendages of the perithecia remain intact and the mode of branching is easily seen. Likewise, the student sees the superficial nature of the mycelium, its tangled condition, and the catenulate manner in which the conidia are borne. For class study of the vegetative parts, *i.e.*, mycelium and conidiophores, clover mildew is better than that found on the lilac because of the more prominent conidiophores with from two to six conidia often remaining attached.

While this method has been developed for the study of powdery mildews, its use may be extended to other superficial fungi or those like the "downy mildews'' in which the conidiophores are exposed. Very satisfactory mounts for study have been made of sooty molds, Trichopelts, Mycrothyriums, and Meliolas. It probably can be used satisfactorily for others of the Hyphomycetes besides the genus Oidium.—HOWARD C. ABBOTT, DEPARTMENT OF BOTANY, UNIVERSITY OF ILLINOIS.

Apple Blotch in New York State.—Apple blotch, caused by *Phyllosticta solitaria* E. and E., was found in April, 1924, in two planting of apple seedlings on a farm at Williamson, N. Y. In July, 1924, Mr. W. O. Gloyer, of the Geneva Experiment Station, reported (letter) the finding of blotch by Mr. Barney Blanch, state nursery inspector, in a nursery at Geneva, N. Y., from which one of the above lots of seedlings was obtained. These seem to constitute the first findings of blotch in New York State. Dr. M. B. Waite, of the U. S. Department of Agriculture, believed (letter to Dr. E. F. Guba, February, 1920) that he found the same fungus on foliage of apple in western New York about 1906, and again in 1910, but later decided that the fungus of those collections was *Coniothyrium pirinum.*

One planting at Williamson was very heavily infected. Eighty-six out of 100 trees were infected and it was estimated that 40 to 50 per cent of these seedlings were heavily cankered on the first year's growth. The spread to the 1923 twigs, however, was relatively slight. Budding on this stock in 1923 was almost a total failure. The relation of the blotch disease to this failure is of course uncertain. Only one plant was found in which the fungus had spread to the scion. On May 16, 1924, a number of trees from this planting were replanted on the University farm at Ithaca, N. Y., in a continuous row along with seedlings which did not show the disease. Five one-year-old trees of standard varieties were interplanted with the diseased trees at intervals of only a few inches. On September 26, eight of the original diseased trees were alive, and two of these showed a slight spread of the canker to the base of the current season's growth. No cankers could be found on the adjacent budded trees or on the seedlings which were clean at the time of planting.

All of the diseased lots of trees mentioned above were purchased from nurseries in Iowa. The practice of purchasing seedlings from the middle west seems to be increasing among our nurserymen. This obviously offers a constant source for importation of the blotch fungus into New York State and one that has undoubtedly existed for a number of years. However, coupled with the above observations, this situation lends support to the view that apple blotch is not likely to become a serious menace under our conditions. Nevertheless, it will be necessary to accumulate a greater body of facts before this or any other view may be considered as established. In spite of the doubt that this pathogene will thrive in this state, the wholesale shipment of heavily-blotched, seedling nursery stock into territory hitherto free from it does not seem to be justified.—H. E. THOMAS, CORNELL UNIVERSITY, ITHACA, N. Y.

Cercospora Leaf Spot of Lettuce.—In Phytopath. **13**: 289, 1923, C. G. Welles describes as new a Cercospora disease of lettuce. He names the pathogene *Cercospora lactucae* and states, "I have searched through all available literature and I fail to find reference to such a Cercospora disease on lettuce." Stevenson (Jour. Dept. Agr. Porto Rico **1**: 105, 1917), described a Cercospora lettuce disease from Porto Rico in 1917. He also proposed the name *Cercospora lactucae,* which fact invalidates *Cercospora lactucae* Welles. The descriptions correspond so closely that they are probably of the same organism. Stevenson's description is much the fuller of the two. The disease, as described by Stevenson, has been called to the attention of American workers by Taubenhaus in his well-known book on vegetable diseases. (Diseases of truck crops, p. 145. New York, 1918.)—FRANK P. MCWHORTER, VIRGINIA TRUCK EXPERIMENT STATION, NORFOLK, VIRGINIA.

PHYTOPATHOLOGY

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DROP OF CHINESE CABBAGE AND OUR COMMON CABBAGE CAUSED BY SCLEROTINIA SCLEROTIORUM (LIB.) MASSEE (SCLEROTINIA LIBERTIANA FCKL.)

W. H. DAVIS

WITH THREE FIGURES IN THE TEXT AND PLATES III TO IV

INTRODUCTION

In the late summer and fall of 1923 and 1924, sclerotia were observed on rotting Chinese cabbage and common cabbage growing in the vegetable gardens of the Massachusetts Agricultural College. The problems which arose while these sclerotia were under observation follow:

1. Are these sclerotia of a pathogenic organism?
2. Is a Botrytis stage associated with the sclerotia?
3. Will cultures of mycelium and ascocarps from these sclerotia show an etiology similar to *Sclerotinia libertiana* Fckl.?
4. What is the specific Latin binomial for the fungus on each host?

THE DISEASE

A description in the literature of this sclerotial disease on Chinese cabbage has not come to the writer's attention. Harter and Jones (7: 24)[1] described the disease on common cabbage as drop (watery soft-rot). In 1924, it was reported from New York and Louisiana as watery soft-rot (9: 197), while Gardner (5: 16) observed a damping-off of cabbage seedlings and a collar-rot of the stem. However, drop of Chinese cabbage seems to be the most suitable common name for this disease since the symptoms are identical with those on other plants infected with the same organism. The following varieties of Chinese cabbage were diseased and under observation: Petsai or Pekin (*Brassica Pe-Tsai* Bailey),[2] Chokurei, Paoting or Wong Bok, Kinshiu and Chosen.[3]

While there were few plants, twenty-four in each varietal row, the loss due to the disease exceeded fifty per cent as the leaves and "leaf-stalks" were generally reduced to rotting masses. Only three of the plants fruited.

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[1] Numbers in parenthesis refer to the reference and page in the literature cited.

[2] Classification of all genera and species of cabbage is that reported by L. H. Bailey, Manual of Cultivated Plants. 1924.

[3] All varietal names for Chinese cabbage and cultivated cabbage are those printed in Simon and Son's 1923 catalog from whom the seeds were ordered.

The disease made its first appearance during the month of September when the lower leaves which were in contact with the soil began to turn brown, "dropped" and finally assumed a shapeless mass. Further examination showed that the petioles and blades were rotted and other leaves attached to the stem directly above "flagged" and appeared water-soaked and sometimes slimy. Mycelium on the surface of these leaves had not made its appearance to the unaided eye and, at first, the disease was thought to be soft rot caused by *Bacillus carotovorus* L. R. Jones. Later, however, white floccose mycelium and sclerotia developed under and between portions of the old rotting leaves (Figs. 1 and 2; Plate IV, no. 4).

FIG. 1. An infected plant of Chinese cabbage. At the base are diseased leaves which have dried. A—A leaf showing "drop." B—White floccose mycelium.

The symptoms in the stems and floral stalks varied. Their interiors were either reduced to a soft rotting mass or turned brown, dried and easily crumbled. Many times the connection between the root and stem was broken at a point just below the surface of the soil where injury was most apparent. Within the dry, hollow, diseased flower-stalks and stems were large patches of mycelium and numerous sclerotia.

Both young and matured plants became infected. In young plants the fungus appeared to advance from the soil to the leaf axils and formed a rapidly spreading water-soaked area along "winged" portions of the blades which are attached to the margins of the petioles. In about 24 hours the infected leaves "dropped" and others above these were likewise attacked. In two cases the stems were entirely rotted and the plant "dropped" in 48 hours.

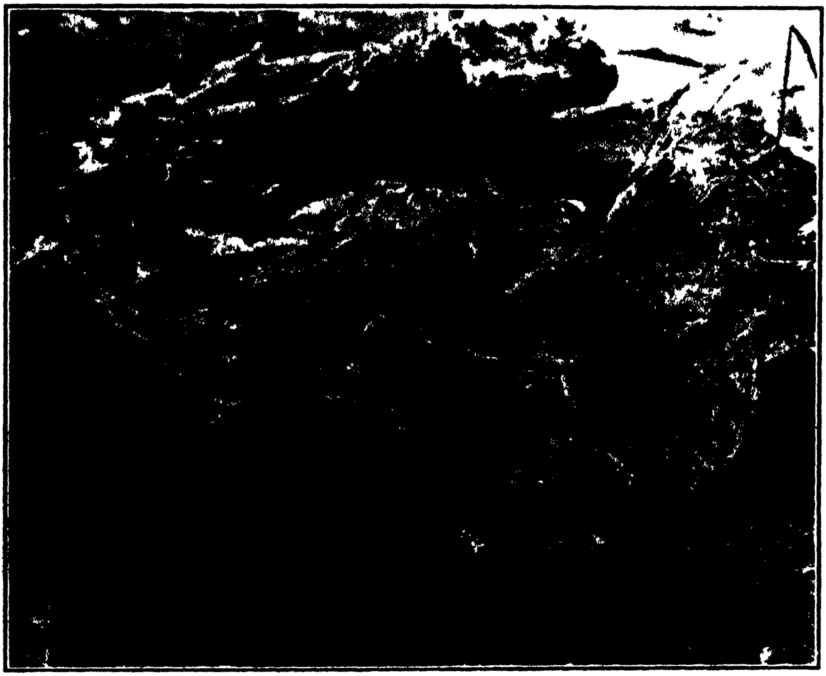

FIG. 2. Photograph of the plant shown in figure 1 taken one month later. The plant has entirely succumbed to the disease. A–B—Large sclerotia.

The symptoms of the disease on cultivated Danish ballhead cabbage (*Brassica oleracea* var. *capitata* L.) have been described by Harter and Jones and, in general, they were similar to those here described for Chinese cabbage. Numerous sclerotia generally appeared on the surface of diseased cabbage heads, but they seldom appeared on the leaf blades and axils within the cabbage head. About ten per cent of the cabbage heads were reduced to a soft rotting mass, while five per cent of the remaining plants were infected. Some diseased cabbage heads turned brown and dried, but retained their shapes while still attached to the roots. Isolations of *B. carotovorus* from the soft-rotting cabbage heads would indicate that this bacillus was acting in conjunction with the Sclerotinia, and the two organisms caused watery soft-rot, while the Sclerotinia alone formed a drop which normally ended in a drying of the cabbage heads without a total disorganization of the plant body (Fig. 3).

THE CAUSAL ORGANISM

Sclerotia: After a heavy frost on November 1, 1923, abundant white tufts of mycelium appeared on the water-soaked areas of leaves, stems, and leaf axils of Chinese cabbage. These tufts varied in size from a millimeter to several centimeters in length, and about one week later they had transformed into white sclerotial bodies. These bodies turned from a cottony-white to a gray and finally to a black color which signified maturity. Matured sclerotia easily separated from the underlying mycelium and fell to the ground, especially when the host materials were disturbed. The sclerotia were irregular in outline and the lower surface lying next to the mycelium and host was concave, while the upper free surface was convex. Under a hand magnifier the surfaces showed many small papillae or mounds, often giving them a botryoidal appearance (Plate III, nos. 4 and 5). Stained sections, when highly magnified, showed an outer rind of from two to five

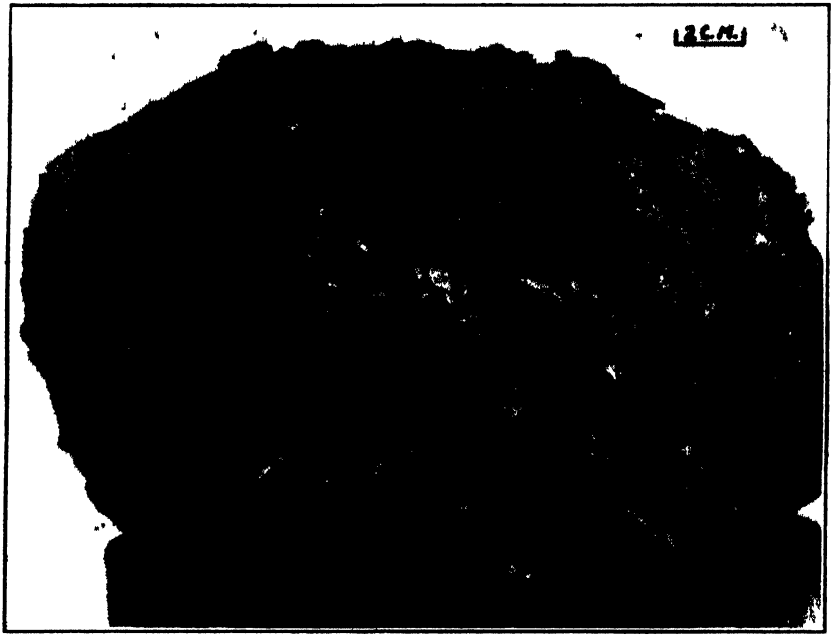

FIG. 3 Sclerotia on a cabbage head.

rows of roundish cells with thick angular walls of an unstained burnt-umber color. The interior was composed of a gelatinous structure of interwoven hyphae, the medulla. Within these hyphae were numerous small, globose bodies which readily stained with gentian violet. The primordia were scattered and near the periphery of the medulla.

Sclerotia varied in size from minute globose or oblong bodies less than 1 mm. to those 3 cms. in length, mostly 5 to 7 mms. (table 1). Under warm, dry conditions, the maximum period of viability was ten months. These sclerotia were collected November 10, 1923, and placed in an open glass jar which was stored in the laboratory. Three of the fifty sclerotia remained viable, bearing ascocarps when placed under the proper conditions. Other sclerotia collected on the same date and stored under the same conditions were placed in moist soil on March 10 and over eighty per cent produced abundant apothecia, thus showing viability after four months storage. Sclerotia stored in damp soil either formed ascocarps early in the following spring or entirely disappeared by the following autumn. This would substantiate the belief that under natural conditions sclerotia do not remain viable near the surface of the soil longer than one year. However, this does not account for those which might have been buried deep in the soil by plowing and returned to the surface by further tillage.

Ascocarps: In November, 1923, abundant matured sclerotia were collected from Chinese cabbage and cultivated cabbage and stored in open glass jars as previously described. In February, 1924, sclerotia from each stored sample were sparsely covered with saturated greenhouse soil placed one inch deep in eight-inch glass moist chambers. These jars were covered, set on a laboratory table near a window in a temperature averaging 20° C., and the soil was kept in a wet condition. On April 15, abundant ascocarps had developed from most of the sclerotia (Plate IV, nos. 1, 2, 3, and 5; Plate III, no. 2). At this time other stored sclerotia were cultured under similar conditions and bore matured ascocarps on June 7. Ascocarps continued to develop in the jars until the following October. Measurements of ascocarps and their parts were made from materials under the following conditions: fresh, preserved in 70 per cent alcohol, and stained sections.

Table 1 shows that the average height of ascocarps was 15 mm. However, these ascocarps were cultured from sclerotia planted just beneath the surface of the soil. No experiments were performed to determine the relation between the height of ascocarps and the depth of sclerotia in the soil.

Near the sclerotia, the stipes were a deep brown which shaded to a chestnut color and finally blended with the light-gray color of the upper stipe and apothecial cups at a point averaging 5 mm. from the sclerotia. As many as 38 ascocarps formed from a single sclerotium.

TABLE 1.—*Measurements of sclerotia, ascocarps, ascospores, asci and paraphyses of Sclerotinia sp. on Chinese cabbage compared with those of Sclerotinia libertiana Fuckel as reported by Saccardo*

		Sclerotia (mms.)	Ascocarps (mms.)	Ascospores (μ)	Asci (μ)	Paraphyses (μ)
Author	(1)	Minute–30	0.3–1 x 10–18	4–6 x 9–11	5– 9 x 100–125	1.5–2.5 x 110–118
	(2)	5–7	0.7 x 15	4.5 x 10.8	9 x 113	2 x 113
Saccardo		To 30		4–6.5 x 8–13	8–10 x 130–135	.

(1) Limits for 50 specimens measured.

(2) Averages for 70 per cent of the specimens measured.

Ascospores from fresh materials averaged 4.5 x 10.8 μ; from materials preserved in 70 per cent alcohol, 4.5 x 9 μ, and in stained sections on prepared slides, 3.5 x 7.2 μ (Plate III, nos. 1 and 3).

The widths of asci varied from 5 to 9 μ; lengths, 100 to 125 μ; average size, 9 x 113 μ. Table 1 shows that these measurements for the widths compare favorably, but the lengths are somewhat less than those reported by Saccardo for *S. libertiana.* The ascospores had not been discharged from most of the asci measured. However, the characteristic "blue tips" were evident. The clavate paraphyses averaged 2 x 113 μ (Plate III, no. 1).

Fresh ascocarps from sclerotia on common cabbage were sectioned and their parts measured. These measurements compared favorably with those reported for Chinese cabbage in table 1, so they were omitted. Thus the measurements of the ascocarps and their parts, except the length of the asci, compared favorably with those reported by Saccardo for *S. libertiana.*

Mycelium was isolated from diseased plants of Chinese cabbage, head lettuce (*Lactuca scariola* var. *capitata* L.), and common cabbage bearing sclerotia. The fungus in each isolation was transferred to potato agar slants and sterile bean pods. With these cultures, inoculations within the three named host species and reciprocal inoculations were made by placing mycelium on the stems and leaves in contact with the soil, in the leaf axils, and on aerial portions, as the upper leaf blades. Both young and old plants of Chinese cabbage, lettuce, and common cabbage were inoculated and the work was performed under greenhouse conditions.

The results showed infections when both reciprocal inoculations and inoculations within the host species were made. Thus, this Sclerotinia on these three hosts possesses one species and no physiological races.

As the leaves bore "target spots," attempts were made to isolate a Botrytis and determine if it was associated with the life cycle of *S. liber-*

tiana. Species belonging to the following genera of fungi were isolated: *Botrytis, Alternaria, Fusarium,* and *Penicillium*. Each fungus was grown in pure culture from single spore isolations, but several inoculations of Chinese cabbage with each culture failed to produce infections. Neither a Botrytis stage nor microconidia were observed in any of the cultures from the sclerotia or the mycelium.

TAXONOMY

According to a fixed custom, early German writers (De Bary, Wakker, Kissling) treated this fungus as *Peziza sclerotiorum* Libert. However, Miss Wakefield (12:126) has shown that Massee formed the new genus Sclerotinia and the specific Latin binominal is *Sclerotinia sclerotiorum* (Lib.) Massee instead of *Sclerotinia libertiana* Fuckel which is generally adopted by our mycologists.

Kissling (8:229) states that Eidam described black bodies on cabbage heads and classified them as *Sclerotinia compactum* DC. Eidam claimed that a Botrytis stage was associated with this fungus. Harter and Jones described drop of cabbage and attributed the cause to *S. libertiana* Fuckel. The writer found no Botrytis stage definitely associated with the life-cycle of this fungus on Chinese cabbage or on common cabbage. The morphology of the organism is, in general, identical with descriptions of *Sclerotinia sclerotiorum* (Lib.) Massee.

TERATOLOGICAL FORMS

De Bary (2:51) observed ''exogenous branches'' of *Peziza sclerotiorum* near the ground and believed them ''purely adventitious, artificially excited or monstrous.'' Worsdell (14) has discussed teratological forms of various fungi and grouped them in seven classes, three of which are: dichotomy, fasciation, and proliferation.

Teratological forms of ascocarps were observed in cultures of sclerotia which had been collected from Chinese cabbage (Plate IV, nos. 3 and 5). Unbranched cylindrical bodies (ascocarps) grew from sclerotia sparsely covered with soil, branched dichotomously at the surface of the soil, and each branch in its early growth appeared to develop normally into an ascocarp averaging 0.5 cm. in diameter at the base, 1 mm. in diameter near the apex, and 1 cm. in height. Later, the apex (apothecial cup) of this primary ascocarp turned a gray-brown color and increased in diameter from 1 to 4 mm. At first it was clavate and then cup-shaped with involute, crenate margins. Between the crenae, secondary ascocarps developed and these varied from 1 to 3 mm. in length and about 0.5 in diameter with as many as fifty individuals from one cup. Tertiary ascocarps developed on the apex of secondary ascocarps and were from 2 to 5 mm. in height, less than 1 mm.

in diameter, and 2 to 5 in number. However, there were variations in branching, number, size, and shape of the primary, secondary, and tertiary ascocarps. The above description is for the majority of the cases examined and may be classed with those due to proliferation.

Several specimens of another type came under observation. Each had an apparently normal apothecial cup, bearing on its upper surface two rows of secondary ascocarps arranged in concentric circles (Plate IV, no. 3).

Free-hand sections of apparently matured primary, secondary, and tertiary ascocarps showed that ascospores were seldom formed. However, one especially well developed secondary ascocarp without tertiaries possessed ascospores. The ends of the hyphae in the apothecial cups were often well developed, clavate, and similar to paraphyses.

Any cause which might be responsible for the formation of these teratological forms would only be speculation since experimental data are not at hand. The hyphae which form the ascocarp develop from the primordia within the sclerotia and are of two types: those which originate in its periphery, the peripheral hyphae, and those which originate above the center, the central hyphae. At first, these two types of hyphae are distinguishable, for the cells of the peripheral hyphae are the longer, but as the ascocarp matures their identity is lost. It is generally believed that the ascogenous hyphae come from the primordia and are the central hyphae. Thus any interruption in the development of the central hyphae would suspend the formation of asci for the time being and perhaps definitely. It would seem that this is what took place; the peripheral hyphae had precedence over the central hyphae which had been delayed in their development for some undetermined cause. However, there yet remains to be explained why the apothecial cup of the primary ascocarp assumed the potentialities of a sclerotium; that is, formed new primordia which would seem necessary for the formation of secondary ascocarps.

Worsdell (14) states that botanists believe abnormal structures are due to one of the following: pure accident; direct action of environment; phylogenetic or hereditary causation.

In this case it would not seem to be due to pure accident for the majority of fifty ascocarps from several sclerotia bore teratological forms.

Environment could not have been the sole factor for producing these forms because normal ascocarps developed in the same cultures simultaneously with teratological forms and in one case apparently from the same sclerotium.

Shall we accept the theory that phylogenetically the ancestors of this Sclerotinia bore apothecia or perhaps perithecia on flattened receptacles not entirely unlike the stromata of *Claviceps purpurea* Tul.?

DISCUSSION

De Bary stated that the ascospores of this fungus germinated at once and formed saprophytic mycelium which remained in the soil during the spring and summer. Parasitic mycelium was formed later in autumn. Poole (10) observed that the infection of celery by this organism under greenhouse conditions occurs almost simultaneously with the formation of apothecia or the development and liberation of ascospores. However, it seems that more observations and experimentations with this fungus should be made before an accurate life history on the different hosts can be stated. However, there is a possibility that the life history of this organism varies according to the host parasitized.

Observations showed that the ascospores from apothecia of this fungus germinated at once, and from two inoculations it was concluded that their germ-tubes would not infect. The fact that the disease appeared late in the autumn instead of early in the spring when apothecia were formed, would also substantiate the fact that the germ-tubes from ascospores do not infect at once. However, buried sclerotia which deep cultivation might have brought to the surface of the soil may have continued to form ascocarps until August. Then the formation of apothecia would have occurred through the summer. Environmental conditions of the hosts might have favored infection, but these conditions are unknown to the writer. Young plants as well as old ones were artificially infected in the greenhouse, and it does not seem probable under the existing field conditions that the old plants were more susceptible than the young.

It was interesting to note the short distances which the mycelium grew over the surface of the soil from the centers of infection; 5 cm. was the longest distance observed. However, the fungus did progress from one plant to another when healthy plants came in contact with the diseased. Thus close planting should be avoided as it aids the dissemination of the organism.

The ability of sclerotia to form mycelium in the spring after passing the dormancy of winter still remains unsolved. Is all the mycelium in all sclerotia destined to form apothecia? A sufficient number of sclerotia should be tested to give definite data, but the writer has found it difficult to culture half a matured sclerotium for viable mycelium and the other half for the formation of apothecia. A "balance" in the sclerotium has been upset and parasitic mycelium might become vegetative under these conditions or *vice versa.* Thus, if the complete life history of this organism should be given at this time, a part of it would be nothing short of speculation.

SUMMARY

1. Sclerotia were collected from diseased Chinese cabbage and common cabbage.

2. Mycelium and apothecia were cultured from sclerotia.

3. Measurements of mycelium, sclerotia, apothecia, asci, ascospores, and paraphyses showed the fungus in general to be identical with *Sclerotinia libertiana* Fuckel, as described by Saccardo.

4. Infections were obtained by inoculating Chinese cabbage, head lettuce, and common cabbage with pure cultures derived by isolating mycelium of Sclerotinia from each host.

5. No physiological races of the fungus on these three hosts were observed.

6. Germ-tubes from ascospores failed to infect the living host tissues.

7. The fungus did not progress on the surface of the soil more than 5 cm. from the center of infection.

8. The disease progressed from infected plants to healthy plants with which they were in contact.

9. The complete life cycle of the fungus is unknown, but observations point toward the ascospores forming saprophytic mycelium, and this in turn forming parasitic mycelium later in the season.

10. No Botrytis stage was found to be associated with this Sclerotinia. The specific Latin binominal for this fungus on Chinese cabbage and common cabbage is *Sclerotinia sclerotiorum* (Lib.) Massee.

11. Teratological forms were observed in which the margins of apothecial cups formed secondary ascocarps by proliferation. Tertiary ascocarps were formed on secondaries.

12. In general, these teratological forms did not bear ascospores.

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PLATE III

Camera lucida drawings of sclerotia, ascocarp, ascus, ascospores, and paraphysis of *Sclerotinia sclerotiorum* (Lib.) Massee from Chinese cabbage.

Number 1. A. Ascus containing eight ascospores.
B. Paraphysis.

Number 2. Outline drawing of an ascocarp from a prepared slide.
A. Hymenium.
B. Sub-hymenium.
C. Stipe.
D. Canal.
E. Point of attachment to the sclerotium.

Number 3. Ascospores stained with Fleming's safranin, gentian violet, orange G stain.

Number 4. Different sizes and shapes of sclerotia.
A. The upper surface showing white mycelium.
B. A black, matured sclerotium.

Number 5. Irregular upper surface (O) of a sclerotium shown in 4B. × 10.

PLATE IV

Symptoms, sclerotia, ascocarps and teratological forms of *Sclerotinia sclerotiorum* (Lib.) Massee on Chinese cabbage and common cabbage.

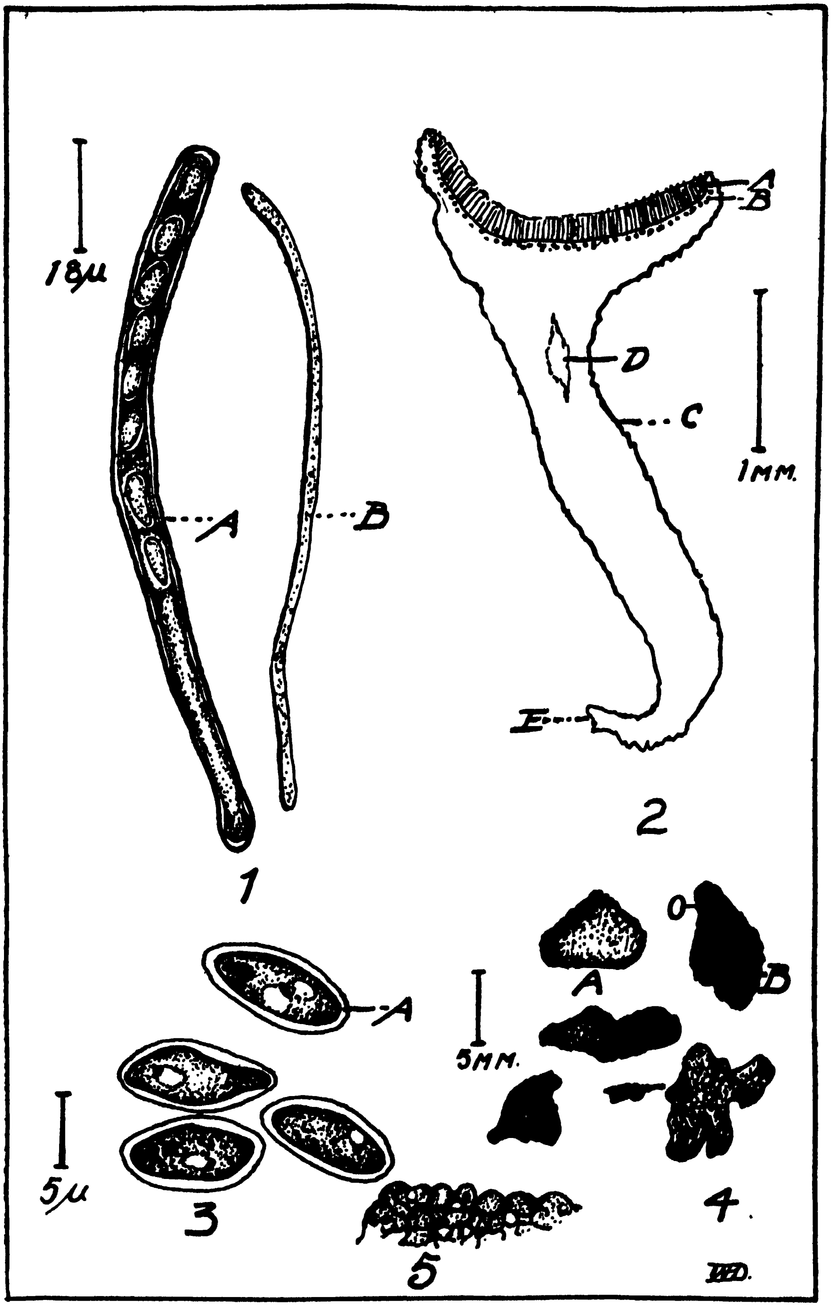
Number 1. Ascocarps of one sclerotium from common cabbage.

Number 2. Ascocarps on a sclerotium from Chinese cabbage.

Number 3. Teratological forms of ascocarps from Chinese cabbage.
A. The secondary ascocarps arranged in two concentric circles.
B. Tertiary ascocarps forming on the apex of secondary ascocarps.

Number 4. Portion of a Chinese cabbage leaf.
A. Sclerotium.
B. Mycelium.

Number 5. Secondary ascocarps forming on the margins of primary apothecial cups. The sclerotia were collected from Chinese cabbage.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ANTHRACNOSE OF EUROPEAN PRIVET

A. J. Mix

With Three Figures in the Text

Anthracnose of European privet, *Ligustrum vulgare* Linn. (more commonly called English privet), was described by Atkinson (1) in 1892. The material which he studied had been received from Penn Yan, New York. He stated that on some of the twigs from twelve to eighteen inches of the terminal growth was dead, and a distinct line was evident between the dead bark and the healthy tissue below. Other twigs with apparently healthy portions bore cankers at a point twelve to eighteen inches from the end. In some cases the canker girdled the twig, causing death of the terminal portion. Acervuli of the causal fungus were evident in the diseased bark of affected twigs.

Atkinson studied and described the fungus. He mentioned its probable relationship with *Gloeosporium fructigenum,* as the fungus causing the bitter rot of apples was then known, but found points of difference which made him decide to call it a new species. The privet fungus thus became known as *Gloeosporium cingulatum* Atkinson. Subsequently Miss Stoneman (5) described the perithecial stage of this fungus, using Atkinson's original cultures and cultures which she had isolated from material received from Manhattan, Kansas. She named the fungus *Gnomoniopsis cingulata.* Incidentally, Miss Stoneman describes a leaf spot bearing acervuli as occurring in some of the privet material received from Kansas. The writer has never observed such a leaf spot.

Von Schrenk and Spaulding (4) found it necessary, on account of priority, to rename the genus *Glomerella,* so that the accepted name of the privet fungus is now *Glomerella cingulata* (Atk.) Spaulding and von Schrenk.

Shear and Miss Wood (3) have shown that the asexual or Gloeosporium stage of *Glomerella cingulata* may occur on a wide range of host plants. It would appear from their work that the fungus on privet is identical with that causing the well known bitter-rot disease of apples.

Although the fungus in question has been the subject of considerable study, there seems to be no later account of this privet disease than that given by Atkinson. This might indicate that it has not caused serious damage over any considerable territory.

OCCURRENCE OF THE DISEASE

The writer's attention was called to this disease in the summer of 1921 by Mr. S. Herbert Hare, of the firm of Hare and Hare, Landscape Architects of Kansas City, Missouri. During the seasons of 1921 to 1924 the disease has caused serious damage to hedges of European privet in the residence section of Kansas City known as the Country Club District. In well established hedges, five years or more old, many plants blighted to the ground, so that part or all of the hedge had to be removed. In some cases where part of the hedge was removed and replanted the replants blighted. The disease is especially serious, as the death of portions of a hedge may necessitate the removal and replacement of the entire hedge, a matter of several years. The destructive effect of the disease on a privet hedge is shown in figure 1.

FIG. 1. A hedge of European privet dying from the effects of the anthracnose.

Subsequent to finding the disease in Kansas City, the writer located several diseased hedges in Lawrence, Kansas. These hedges have since died. In 1924 specimens of the disease were received from Bartlesville, Oklahoma. It is not known in what other localities the disease may occur, as no search has been made.

The anthracnose has been found only on European privet, and on a privet called Golden Russian by Mr. Hare. A hedge of this privet, planted by Mr. Hare in the spring of 1921, died that same season. Hedges of other privets, Amur, Ibota, Regel, and California, are common in the localities mentioned, but no case of the disease has ever been found in any of them.

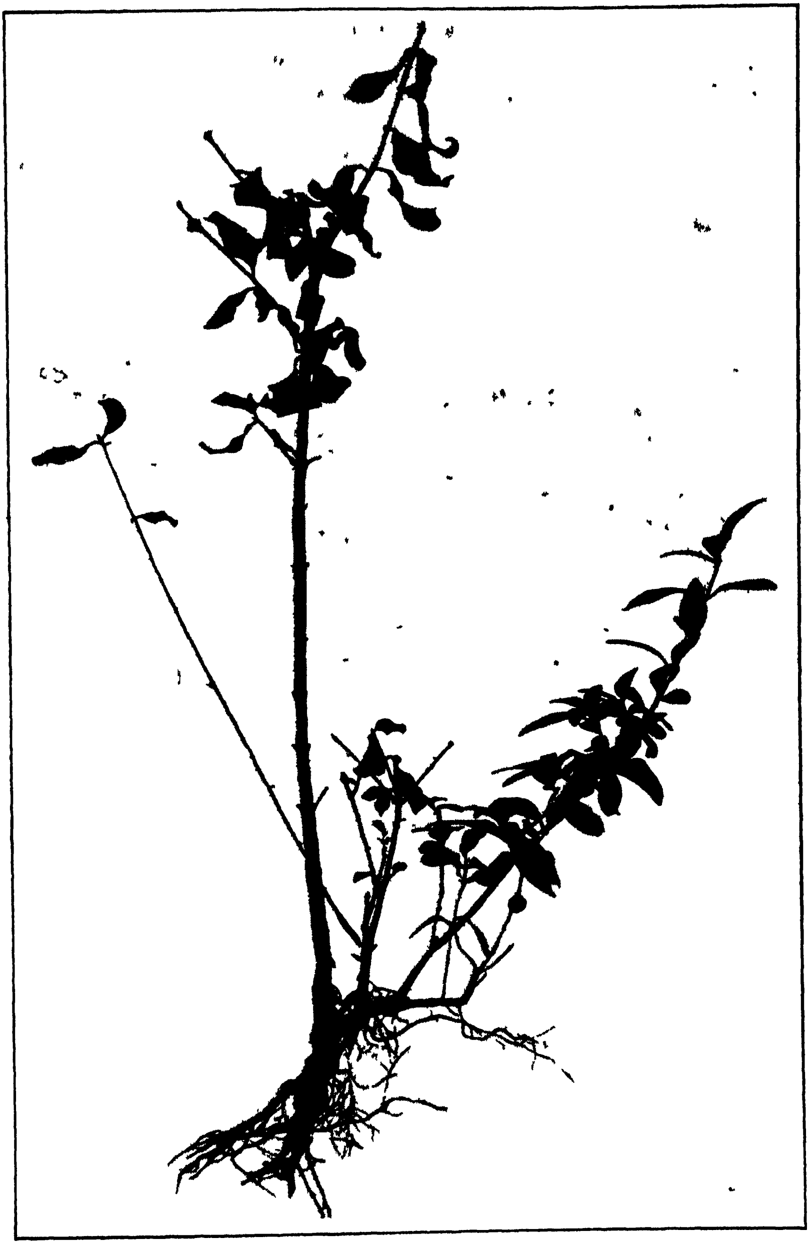

FIG. 2. A plant of European privet dying as the result of artificial inoculation with *Glomerella cingulata*. The notch in the stem below the ground line indicates the place of inoculation. The branch to the right has remained healthy.

DESCRIPTION OF THE DISEASE

Symptoms of the disease, as observed in Lawrence and Kansas City, are somewhat different from those described by Atkinson. The twig blight and twig canker have been frequently found and these manifestations of the disease accord with his descriptions. More commonly the whole plant is affected. Such a plant shows dead dry leaves which cling persistently and turn first pale green and later brown. The bark and wood of the upper portions of the plant remain bright colored until after the death of the leaves, later appearing brown and dead, but free from fungous invasion. The appearance of a dying plant is shown in figure 2, which, however, represents a plant which had been artificially inoculated.

Examination of the basal portion of a diseased plant reveals one or more cankers on the main stem just above the ground line or even below it. These cankers are at first long-oval in outline but eventually they enlarge and coalesce until the stem is girdled. The diseased bark is brown and frequently shows acervuli of the fungus. A dead stub is usually evident in the canker, indicating that the fungus entered by way of a blighted basal shoot. The wood beneath a canker is discolored brown or grayish black. This discoloration may extend only a little way into the wood or may involve the whole interior of the stem, depending on the age of the canker. Typical cankers and the discoloration of the subjacent wood are shown in figure 3.

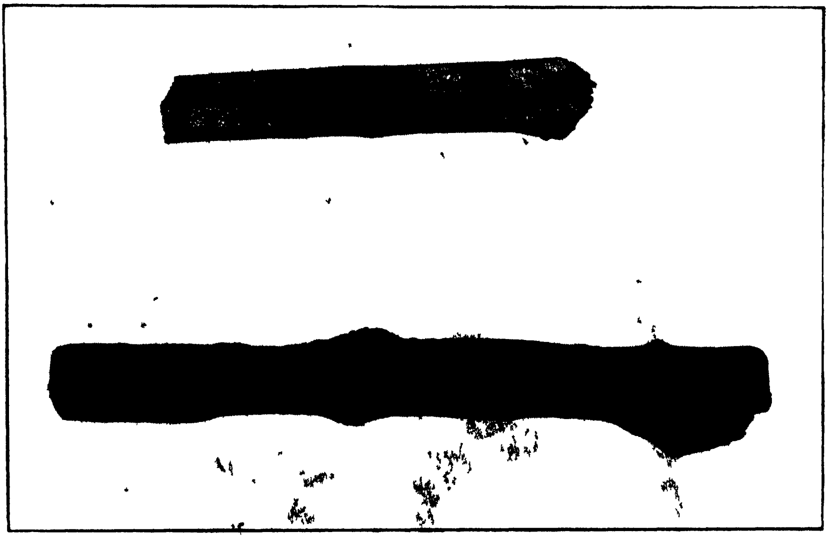

FIG. 3. Stem cankers caused by the anthracnose fungus. Dead stubs indicate the place of entrance of the organism. The split stem shows the typical discoloration of the wood beneath a canker.

Microscopic examination of sections of cankers shows that all the tissues of the bark are brown and dead. Mycelium of the fungus may be observed between or within the cells of all tissues except the bast fibers. In the wood the mycelium is never abundant, but hyphae may be seen here and there within the cells of the xylem and medullary rays, and are most readily seen crossing or extending up and down the tracheal tubes. There is no disintegration of the tissues of the wood until late stages of the disease when the wood has apparently become invaded by other fungi.

Although the mycelium is common in the tracheal tubes, there is no evidence that its presence there is directly responsible for the death of the plant, as is the case with wilt-diseases of other plants. Apparently the plant dies only after the bark and wood of a portion of the stem have been killed. This may not occur the same season as infection, but often the second or third season thereafter. Sometimes one or more branches die the first year, and the rest of the plant the year following.

The roots are apparently not infected, at least so long as the plant is alive. Isolations from old decaying roots of dead plants have yielded a variety of organisms but not the anthracnose fungus.

These observations show that this privet disease is more destructive than is indicated by Atkinson's description. As he dealt only with material which had been sent to him from a distance, it is possible that he was unfamiliar with the more severe manifestations of the disease. Again it is possible that it is more destructive in the middle west than in the east. As observed here the disease would be aptly characterized by the term privet blight, but it seems unwise to substitute this name for the well established, though less appropriate one of privet anthracnose.

The disease usually becomes evident in late June or early July, and the number of dead or dying plants gradually increases throughout the season. The incidence of the disease corresponds closely to the coming of hot weather, and seasons in which the disease has been especially destructive have been rather hot and rainy.

ISOLATIONS

Isolations from the basal portions of diseased plants were made at different times during 1921, 1922, and 1923. Fragments of wood were removed, under aseptic conditions, from the interior of diseased stems and placed in tubes of potato dextrose agar. Isolations were made from twenty-two plants, two of which were Golden Russian privet, the remainder were European. The results may be summarized as follows: total number of tubes, 151; *Glomerella cingulata,* 117; *Verticillium* sp., 5; miscellaneous undetermined fungi, 16; blank, 13. One set of poured-plate isolations which was made from acervuli on diseased twigs yielded pure cultures of *Glomerella cingulata.*

BEHAVIOR OF THE FUNGUS IN CULTURE

Single-spore strains of the fungus were not employed for the cultural studies, but separate cultures, each arising from a separate isolation, were used. No extensive study of cultural relations was attempted, inasmuch as the important facts regarding the behavior of *Glomerella cingulata* in culture are already well known.

The fungus was grown on a number of common media, including potato dextrose agar, corn meal agar, oat agar, corn meal, and rice. Some variation in character and amount of growth on different media was observed, but none worth reporting. Corn meal agar proved to be the best medium for the formation of perithecia.

On potato dextrose agar the fungus grows rapidly, producing an abundant aerial mycelium. Considerable variation was noticed between different cultures as to the color of this mycelium, it varying from deep gray to grayish white or nearly pure white. On corn meal agar and on all agars containing dextrose, certain cultures produce a red pigment which diffuses into the agar. In other cultures this pigment is lacking.

In most cultures black stromata appear in about a week. These are scattered without order over the surface of the agar. The characteristic oblong conidia of the fungus are borne in these stromata or on the tips of long aerial hyphae. In some cultures the formation of stromata and conidia is almost entirely suppressed; such cultures, when older, produce perithecia abundantly.

Immature perithecia have been found in cultures two weeks old, and mature ascospores in cultures six weeks old. The perithecia and ascospores agree with the descriptions given for this fungus by Miss Stoneman (5) and others.

It has been mentioned that corn meal agar is a favorable medium for the production of perithecia, but, as Shear and Miss Wood (3) have pointed out, the ability to form perithecia seems to be an inherent quality of a particular strain of the fungus rather than something induced by the culture medium. Of twenty-two cultures, each representing a separate isolation of the fungus grown on corn meal agar, fifteen produced perithecia. In seven of these cultures mature ascospores formed before the end of twelve weeks, and in seven cultures no perithecia were found.

INOCULATIONS

The objects of the inoculation experiments were: (1) to establish the pathogenicity of the fungus for European privet; (2) to see if the observed immunity of the other privets held true; (3) to determine if spores of the fungus could enter uninjured twigs; (4) to study possible relations between *Glomerella cingulata* from privet and the same fungus from apple.

For purposes of inoculation a number of young plants (apparently two years old) were obtained in March, 1922, and divided into two lots. One lot was planted in the greenhouse, the other out of doors. The following privets were represented: European, Amur, Ibota, Regel, and California.

Inoculations were made on the indoor plants on July 3. The dirt was scraped away from the base of a plant and an incision made in the stem with a sterilized scalpel. Mycelium and agar from a seven-day culture were placed in the incision and the dirt replaced. Similar incisions were made in the stems of the plants used as controls, but no mycelium was inserted. Control plants were in a part of the bed distant from the inoculated plants. Of the four inoculated plants of European privet, one was entirely dead at the end of two months and another was dying. A photograph of this dying plant is reproduced in figure 2. A third plant was found diseased at the end of three months and this and the fourth plant were entirely dead in six months from the time of inoculation. The one European plant used as a control remained healthy. The appearance of the diseased plants was typical of the disease as observed under natural conditions, and the usual discoloration of the wood was found around the point of inoculation. The fungus was reisolated from the discolored stems of each of these diseased plants.

Inoculated plants of the other privets: four plants each of Amur and California, and three each of Ibota and Regel remained healthy, and at the end of six months the incisions in their stems had healed. The same was true of the control plants: two plants of Regel and one each of the other privets.

Four plants of European privet growing out of doors were inoculated in a similar manner on July 19. Two months later a main branch of one plant was dead to its base, but the rest of the plant appeared healthy. The fungus was recovered from the lower part of the diseased branch. This plant died early the following year, showing typical symptoms of anthracnose. Of the remaining plants, one was observed to be diseased at the base in July, 1923. This and a third plant died late that summer. The fourth plant appeared healthy until 1924 when it also died. One plant used as a control remained healthy for two years, dying in 1924 as the result of later inoculation.

Inoculations were also made on four plants each of Ibota and Regel privets, on three plants of California, and two of Amur. One plant of each was used as a control. All inoculated and control plants remained healthy.

A number of inoculations were made in twigs of privet plants growing out of doors. A slit was made in the bark by means of a sterilized scalpel and inoculum inserted. The wounds were left unprotected. Similar incisions, to serve as controls, were made in other twigs, but no inoculum was inserted. The results of these inoculations are summarized in table 1. The infections recorded in this table consisted in a few cases of small cankers around the incisions; but, in most cases, a die-back of the twig resulted which extended considerably below the point of inoculation. Acervuli of the fungus were evident on many of the diseased twigs. The two infections of control twigs noted in the table were small cankers formed around the incisions. Evidently these resulted from natural inoculation.

The inoculated twigs were too small for easy removal of diseased wood under aseptic conditions. Reisolations were therefore attempted in only a few cases. These resulted in the recovery of the organism from six of the inoculated twigs.

TABLE 1.—*Results of inoculating privet twigs with Glomerella cingulata*

Variety	Part inoculated	Date	Inoculations	Infections	Control incisions	Infections of controls
European	One-year wood	May 1, 1923	10	9	6	0
do	Two-year wood	do	5	5	3	0
do	One-year wood	Aug. 1, 1923	10	10	6	2
Amur	do	do	10	0	6	0
Ibota	do	do	10	0	6	0
Regel	do	do	10	0	6	0
California	do	do	10	0	6	0
European	do	July 4, 1924	7[a]	6	3	0
do	Two-year wood	do	5[a]	4	3	0
do	One-year wood	do	6	6	—	—
do	Two-year wood	do	6	6	—	—
Amur	One-year wood	do	6	0	—	—
do	Two-year wood	do	6	0	—	—
Ibota	One-year wood	do	6	0	—	—
do	Two-year wood	do	6	0	—	—
Regel	One-year wood	do	6	0	—	—
do	Two-year wood	do	6	0	—	—

[a] Inoculated with a culture of *G. cingulata* from apple fruit.

INOCULATIONS OF UNWOUNDED TWIGS

Observations of the disease in nature indicate that the fungus can enter the unwounded tips of twigs. The basal cankers already described apparently originate from basal shoots thus infected or from infection of undeveloped lateral buds.

On August 7, 1923, several inches of the ends of eight growing shoots of European privet were sprayed with a water-suspension of spores and mycelial fragments from culture. Two other shoots were sprayed with water for controls. All shoots were inserted in lamp chimneys plugged at both ends with cotton and left thus covered for forty-eight hours. Four f the inoculated shoots developed die-back, while the other four and the controls remained healthy. On two of the inoculated shoots small cankers developed some distance back of the tip, indicating that infection may occur in unwounded bark of the current season's growth.

CROSS INOCULATIONS WITH PRIVET AND APPLE

As is indicated in table 1, some of the inoculations of privet were performed with a culture of *Glomerella cingulata* from apple. As apple bitter rot is very rare in this part of Kansas, no culture was at hand. The one used was kindly furnished by Dr. H. W. Anderson, who isolated it from apple fruit in Illinois.

On July 4, 1924, a number of inoculations were made into twigs of Ben Davis and Missouri Pippin apple trees, using the culture of *Glomerella cingulata* from apple and several cultures of the privet fungus. No infections resulted from these inoculations. It seems unwise to delay publication of this article to secure further data on this point. Shear and Miss Wood (3) present a summary of the cross inoculations made with *Glomerella cingulata* by them and by previous investigators. They record no privet-apple cross inoculations.

INOCULATIONS OF APPLE FRUITS

Inoculations of ripe apple fruits were made, using several cultures of the fungus from privet and the apple culture mentioned above. The fruits were surface-sterilized with mercuric chloride, 1 to 1,000, and allowed to dry. A wedge-shaped piece of tissue was removed from each fruit with a sterilized scalpel; inoculum, consisting of mycelium and agar from a culture of *Glomerella cingulata*, was placed in the well thus formed, and the wedge of tissue replaced. The fruits were then covered with bell jars. Control fruits were similarly treated except for the insertion of inoculum. Only a few cases of rot developed in the controls, and in these cases some other organism than *Glomerella cingulata* was responsible.

The results of fruit inoculations (omitting the control fruits) are summarized in table 2. Observations were made when any fruit had reached a stage of total decay. This condition is indicated in the column headed "infection" by six plus-marks. The relative rate of decay of other fruits is indicated by a smaller number of plus-marks. A plus-mark in the column headed "reisolations" indicates that planting bits of decayed tissue in tubes of potato dextrose agar resulted in pure cultures of *Glomerella cingulata*. It is evident from this table that some cultures of *Glomerella cingulata* from privet can cause decay of ripe apples as readily as the apple-strain of the fungus, others much less readily or not at all.

TABLE 2.—*Results of inoculating apple fruits with pure cultures of Glomerella cingulata*

Variety	Culture	Source	Infection	Reisolations
Blacktwig	27	Privet	++++++	+
"	32	"	++++++	+
"	34	"	+	+
"	9	"	+	+
"	33	Apple	++	+
Jonathan	27	Privet	++++++	+
"	9	"	+	+
"	29	"	++++++	+
"	39	"	++++++	*Alternaria* sp.
"	35	"	+++	+
"	42	"	+	*Penicillium* sp.
"	38	"	+	....
"	17	"	0	....
"	12	"	0	....
"	33	Apple	++++++	+
Grimes	27	Privet	+	+
"	9	"	0	....
"	29	"	++++++	+
"	39	"	++++++	+
"	35	"	+	*Alternaria* sp.
"	42	"	0	....
"	38	"	++++++	+
"	17	"	0	....
"	12	"	0	....
"	33	Apple	++++++	+
York Imperial	9	Privet	+	+
" "	29	"	++++++	+
" "	39	"	+	*Alternaria* sp.
" "	35	"	+	+
" "	42	"	+	+
" "	12	"	0	....
" "	33	Apple	+++	+
Ben Davis	27	Privet	++++++	+
" "	9	"	+	....
" "	29	"	++++++	+
" "	39	"	++++++	+
" "	35	"	+	....
" "	42	"	+	+
" "	38	"	++	+
" "	12	"	0	....
" "	19	"	0	....
" "	33	Apple	++++	+

CONTROL

It seems possible that partial control of this disease might be accomplished by spraying. As, however, the use of privet as a hedge plant renders anything short of complete control valueless, no spraying experiments have been attempted.

It seems best to recommend that where the anthracnose is found to be serious other privets be substituted for European (English) privet. The record of observations and of artificial inoculations reported earlier in this paper indicates that several privets are immune to anthracnose. There is nothing surprising in this immunity as the various privets under consideration are, with one exception, separate species.

The following is a list of the privets most commonly used for ornamental planting. The nomenclature is that of Olmstead, Coville, and Kelsey (2).

Ligustrum vulgare. European privet.
Ligustrum amurense. Amur privet.
Ligustrum ibota. Ibota privet.
Ligustrum ibota regelianum. Regel privet.
Ligustrum ovalifolium. California privet.

European privet is the only one of these susceptible to anthracnose. California privet often winter-kills in this climate, but the others are hardy. Amur and Ibota privets make excellent hedges and seem acceptable substitutes for European privet, at least in Kansas and neighboring states.

SUMMARY

Anthracnose of European (English) privet, caused by the imperfect stage of *Glomerella cingulata,* was first described by Atkinson in 1892.

During the past few years this disease has been observed to be destructive to privet hedges in Kansas City, Missouri, and vicinity.

In addition to the twig-blight and twig-canker seen by Atkinson, girdling cankers at the base of the plant have been found. Such cankers cause the death of the plant, although not always in the same season that infection occurs. The disease is therefore more destructive than is indicated by Atkinson's description.

Isolations from diseased plants have yielded, in most cases, pure cultures of *G. cingulata.*

The fungus agrees in cultural characters and behavior with descriptions given by other investigators.

Wound-inoculations into main stems and twigs of privet plants have shown the fungus to be pathogenic for European privet, but not for Amur, Ibota, Regel, and California privets.

The fungus has been reisolated from inoculated plants of European privet.

One inoculation experiment indicated that spores of the fungus may enter the unwounded tips of growing twigs. Such entrance apparently occurs in nature.

Positive results were obtained when twigs of European privet were inoculated with a culture of *G. cingulata* from apple. One attempt to inoculate apple branches with cultures from both apple and privet failed.

Inoculations into apple fruits indicated that some strains of *G. cingulata* from privet decay apples as readily as does *G. cingulata* from apple, others less readily, and others not at all.

The only feasible remedy for the disease seems to be the substitution of another privet for European privet. Hedge privets suggested for the middle west are Ibota and Amur.

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FLAGELLATES IN PLANTS: A REVIEW OF FOREIGN LITERATURE

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HISTORY

A Leptomonas, which is a flagellate of the family of the trypanosomides, was found in April, 1909, in the latex of three species of Euphorbia by Dr. Lafont (19), then director of the Microbiological Laboratory in Mauritius. This discovery aroused general interest among protozoologists and specialists in human and animal flagellosis. Lafont named the flagellate *Leptomonas davidi* n. sp. in honor of his assistant David who was the first actually to see the organism. *L. davidi* was reported from several tropical countries soon after Lafont's first publication. In 1911 Dr. Carlos França (6), medical officer and eminent Portuguese protozoologist, found the same flagellate for the first time in Europe parasitizing two species of Euphorbia commonly found in the neighborhood of Lisbon. Euphorbiae invaded by *L. davidi* have been found more recently in Italy (32, 23, 24), Sicily (32), and France (33). In 1916 and 1920 Dr. Migone found two more species of Leptomonas in the latex of milk weeds from Paraguay (27, 9). Finally in a number of hasty preliminary notes (11–18), Dr. Franchini, an Italian physician, now working at the Pasteur Institute at Paris, has described a series of flagellates and amoebae as parasitizing a large number of laticiferous plants, most of them tropical, growing in botanical gardens, either in greenhouses or out of doors.

As can be seen from this brief survey, the presence of flagellates as plant parasites has been reported by a number of authors from many localities, and in several hosts. A considerable amount of work has already been done in this interesting new field, yet, astonishing as it may seem, no contributions have been made by plant pathologists, and, moreover, until very recently the whole matter seems to have been ignored by botanists. The first mention of these plant parasites that we have been able to find in any publication dealing with plant life is the excellent paper by Mesnil (26) in 1921. Since then this subject has been reviewed in the Zeitschrift für Pflanzenkrankheiten by Nieschulz (28) and in the Review of Applied Mycology (1, 2).

FLAGELLOSIS OF EUPHORBIAE

Host range and geographical distribution. A list of the known hosts of *L. davidi*, and the names of localities from which flagellosis has been reported appear in table 1.

TABLE 1.—*Host range and geographical distribution of Leptomonas davidi*

Hosts	Localities from which flagellosis has been reported
Euphorbia pilulifera	Mauritius (19), Madagascar, Mayottes, Madras (4), Zanzibar, Dahomey (3), Senegal (25), Martinique (29), and New Caledonia (26).
E. thymifolia	Mauritius (20), Madagascar, and Mayottes (4).
E. hypericifolia	Mauritius (21), and Madagascar.
E. indica	Belgian Congo (30).
E. peploides	Algeria (31).
E. peplus	Portugal (5), Italy (23) and Sicily (24). (Not infected in Mauritius.)
E. segetalis	Portugal (5), and Italy (32).
E. dulcis, E. falcata, E. nereifolia, E. virosa, and *E. humifusa*	Italy (23).
E. grandis	Sicily (23).
E. schimperiana, and *E. cuponi*	Sardinia (32).
E. helioscopia, L., *E. esula,* and var. *mosana* DC.	France (33).

Symptoms. The different authors do not agree concerning the effect produced on the host by the presence of Leptomonas. Lafont (20) and França (7, 8) believe that the flagellates produce a real disease. Other authors, however, consider the parasite entirely innocuous. Lafont states that in Mauritius parasitized specimens of *E. pilulifera,* for example, can be easily recognized by their debilitated aspect followed by a wilting and yellowing of the leaves. When completely withered, the stems of parasitized plants become thin, brown, and very brittle. França (8) describes similar symptoms for *E. peplus* and *E. segetalis* and also lesions formed around the insect puncture through which the parasites presumably first entered the plant. França (7, 8) confirms Lafont in stating that the presence of the flagellates in the laticiferous tubes may either be general or else strictly localized. In some cases localization is such that a single leaf from a twig may be parasitized while the others are not invaded. França has described and figured the stunting effects of what he calls a heavy infection on growing shoots and the recovery of the plant in cases of light infection. Bouet and Roubaud (3), Rodhain and Bequaert (30), Noc and Stevnel (29), and Léger (25), affirm in contradiction to the above mentioned authors that it is impossible to distinguish invaded from non-invaded plants without a microscopical examination. The contradictory results of authors working in different localities might very possibly be explained by a careful study of symptomology under varied environmental conditions.

Pathological histology. Studies of pathological histology have been reported by França (8). Flagellates are first found in the necrotic tissue around the insect puncture which the author calls the "primary chancre." Except for this localized region, flagellates are not found outside the laticiferous tubes. The invaded tubes are ordinarily few in number. When invaded, however, they usually contain great numbers of parasites. A clogging of the tubes is often produced due not only to the number of flagellates present but also to the coagulation of the latex. França thinks that the independence of the laticiferous tubes from each other throughout the plant, as well as this process of clogging, might explain to a certain extent the surprising localization of the flagellosis.

According to França the presence of numerous flagellates in the laticiferous tubes gradually leads to a disappearance of starch grains, not only from the latex, which becomes watery and colorless, but also from the neighboring parenchyma cells. The chlorophyll bodies themselves may eventually disappear. In the primary lesion a very marked alteration of the tissues can be seen. There is a dark center of necrotic tissue and beneath this a band of corky cells is often found.

THE CAUSAL ORGANISM: LEPTOMONAS DAVIDI LAFONT

Morphology. Lafont's organism, to which he assigns the binomial *Leptomonas davidi,* is polymorphic, like all trypanosomides. The typical form most commonly found in a smear or hanging drop of invaded latex is an elongated flagellate 16 to 20 microns long and 1.5 to 2 microns wide. The body tapers gradually toward the anterior extremity and is flattened out like a ribbon and twisted two or three times around its axis at the posterior end. In fixed and stained preparations a nucleus with a membrane and small granules of chromatin can easily be detected one third of the total length back from the anterior extremity. A second chromatic body of dense structure and smaller dimension is the kinetonucleus or blepharoplast, found in the proximity of the anterior extremity, from which arises a flagellum 10.5 to 16.5 microns long. In its general structure the organism resembles a simple stagnant water flagellate, such as Cercomonas. The presence of the kinetonucleus at the base of the flagellum, and the absence of a digestive vacuole, however, place it among the parasitic flagellates of the family of the trypanosomides. All members of this family known hitherto are parasitic in the digestive tract of invertebrates (mostly insects) and in the blood of vertebrates.

The multiplication of the parasite in the latex is accomplished by means of a longitudinal binary cleavage. The blepharoplast divides first producing two granules, one of which retains the old flagellum, while a new flagellum buds from the other granule (7, 8). The nucleus divides next, and finally the body splits, beginning at the anterior extremity.

Cultures. Attempts to culture the organism have not been successful. On starchy and sugary media, cysts are produced which burst open, and aberrant-looking flagellates are liberated. On extracted latex and on latex agar the organism lives 24 to 48 hours. Flagellates have been cultured for 16 days on the medium of Nicolle (blood agar), and multiplication by binary fission was observed. These colonies, however, died when transferred.

Experiments on the transmission of flagellosis by insects. So striking is the resemblance of the flagellates found in the latex of the Euphorbiae to those commonly parasitic in the digestive tract of insects that from the very first all students of plant flagellosis have searched for the alternate host and transmitting agent of *L. davidi* among the insects commonly found on Euphorbiae. Lafont (22) attempted to transmit the infection by means of the puncture of *Nysius euphorbiae* Horvath, a hemipterous insect feeding exclusively on Euphorbiae. Having allowed a number of starved individuals to feed on heavily infected plants, he succeeded in transmitting the infection to healthy branches of an infected plant and also to an entirely healthy one. Bouet and Roubaud (3), working in Dahomey, found flagellates resembling *L. davidi* in the intestine of another latex-sucking hemipterous insect (*Dieuches humilis* Reuter). Eighty starved Dieuches were fed on heavily infected plants for one week and then placed on six healthy plants for about another week. Twenty days later one of the plants was found parasitized. The authors claim that *D. humilis* is capable of transmitting the infection and is most probably the insect host of *L. davidi*.

França (7), in his publication of 1914, expressed the opinion that the real insect host, the natural source of infection of the Euphorbiae, had not yet been found, no worker having been able to find parasites in the salivary glands of an insect, or to produce infection by punctures from insects found in nature. Later França himself (8) found the insect which proved to be, in Portugal at least, the real alternate host of *L. davidi*. He has been able to produce infection with insects found in nature and to study the complete life cycle of the flagellate both in the plant and in the insect.

The alternate host of L. davidi. The insect host of *L. davidi* in Portugal is a small coreid, *Stenocephalus agilis* Scop. This insect is particulary difficult to observe because of its nocturnal habits. França noticed that flagellates resembling *L. davidi* were often found in the digestive tract of this hemipteran. He then discovered that on plots of healthy Euphorbiae no parasitized insects were collected, whereas a high percentage of infected Stenocephali were found in localities where flagellosis prevailed. This author has studied infected specimens found in nature as well as Stenocephali fed experimentally on parasitized latex. In this manner he was able to establish the fact that the flagellates found in the intestine of naturally infected insects are identical with those found in the gut of experimentally infected ones.

Development of L. davidi in the insect host. Having captured Stenocephali in localities where flagellosis of Euphorbiae had never been observed, França (8) allowed them to feed on heavily parasitized plants and then killed and examined them. He thus obtained the principal stages in the evolution of the parasite in the insect host. The flagellates found in the stomach of *Stenocephalus agilis* on the first, second, and third days after ingestion of parasitized latex are in active division, by means of binary or multiple cleavage. After the third day, França finds, among others, large multi-nucleated forms that seem to precede schizogony. These multinucleated forms are probably preceded by isogamic conjugation. França figures what he supposes to be a stage in one such process of conjugation. Very small parasites are found about the eighth day all along the intestine, particularly in the mid-gut and also in the salivary glands. These measure from 4.5 to 7 microns long by 0.8 to 1.5 microns wide. Many of them are devoid of a flagellum. These forms are produced in great numbers. When colored by the usual Giemsa method the nucleus and blepharoplast stain intensely, whereas the protoplasm remains very pale. These are, according to França, the really infective forms in the life cycle of *L. davidi.* They are found in the salivary glands of the insect, as well as in the tissue of the plant in the neighborhood of the insect puncture. França, very properly, calls these small forms "metacyclic," because they constitute the link between the life cycle of *L. davidi* in the insect and the life cycle in Euphoribia.

Overwintering. In the mild climates in which flagellosis of Euphorbiae has been found, Leptomonas lives during the winter in the latex of a certain number of plants (8). These plants constitute in spring the source of infection for newly hatched Stenocephali. On the other hand, it is reported that insects having contracted the infection one season are capable of harboring *L. davidi* during the winter. On one occasion, for example, França (8) found the salivary glands of two insects just coming out of hibernation practically swarming with metacyclic forms of quite normal appearance.

Influence of environmental conditions. All recorded observations seem to indicate that warm and dry conditions are necessary to the development of flagellosis of Euphorbiae.

Single or multiple origin of L. davidi. It is not possible on present evidence to state whether the Leptomonas of Euphorbiae found in different parts of the world belong to one species or whether they are simply forms of convergence of several species of insect Leptomonas adapted to the latex of Euphorbiae. It is theoretically difficult to conceive how convergence could produce forms not only similar, but apparently identical, as those found by Lafont and França (8). If, however, *L. davidi* is primarily an insect parasite, as seems probable, and if Stenocephalus, the primitive host of *L. davidi* in Portugal, does not exist in tropical countries, as it has been claimed, we shall be forced to believe that the same species of Leptomonas can parasitize several genera of insects, or else that different strains of *L. davidi* are of different origin. Laveran and Franchini (23) bring experimental evidence that seems to indicate that insect flagellates, when introduced into the latex of Euphorbiae, not only live in this new medium, but have the tendency to become similar to *L. davidi.* These authors claim to have actually produced typical flagellosis by injecting a pure culture of an intestinal parasite of the dog flea (*Herpetomonas Stenocephali*) into several specimens of two species of Euphorbiae. The authors claim that flagellates recovered from the latex of the inoculated plants had become different from those in the pure cultures, having acquired the twisted bodies characteristic of all plant Leptomonas known hitherto.

FLAGELLOSIS OF SOUTH AMERICAN MILK WEEDS

In 1916 Dr. Migone (27) found a Leptomonas in the latex of a climbing milk weed (*Araujia angustifolia*) growing in the marshes of Paraguay. All the plants growing in one locality were found to be parasitized, yet presented no symptoms of disease. The flagellates gradually disappeared from the latex of specimens removed to another part of the country. The new flagellate was named by Migone *Leptomonas elmassiani* n. sp. This protozöon is so transparent that its presence in a drop of latex can be detected only by the movement of the starchy granules. Colored slides show that *L. elmassiani* resembles *L. davidi* in structure, but is usually smaller. Dr. Migone succeeded in culturing the parasite on blood agar. Little is known concerning the life cycle and transmission of the flagellate. *Onchepeltus lactuosus* is the only insect that was ever found feeding on the plants. In the gut of this Hemipteran, Migone observed round parasites, containing a nucleus and a blepharoplast, which might very well belong to the life cycle of *L. elmassiani.*

In 1920 Dr. Migone found a new Leptomonas parasitizing another milk weed (*Morrenia odorata*). In this case also the flagellate appeared to be harmless to the plant. Material was sent by Dr. Migone to França, who has described the parasite and named it *Leptomonas bordasi* n. sp. (9). The organism resembles *L. davidi*, but is slightly larger.

FLAGELLATES AND AMOEBAE FOUND IN LATICIFEROUS PLANTS

Franchini has found with astonishing frequency parasitic protozoa in a large number of laticiferous plants belonging to several different families. Beyond the statement that heavily infected plants are usually yellow and poor in latex, he says very little of the appearance of the invaded plants (14). The author has studied mainly smears of latex. In a few plants, however, cross sections of fruits, leaves, and twigs were made, and stages of multiplication of the parasites inside cells of different tissues were found (14, 16, 18). From the latex the author describes small Trypanosomes and Herpetomonas (=Leptomonas) 8 to 20 microns long, extremely variable in size, and, to judge by the hasty sketches, often very aberrant in shape. These flagellates show marked amoeboid movements and curious involution forms, particularly under adverse conditions such as low temperature (11). In the latex of most plants he finds also amoebae of different sizes, varying considerably in structure from one host to another.

By using Noeller's blood agar, Franchini has succeeded in culturing parasites of *Chlorocon whitei* (17), *Ficus parietalis* (18), *Ficus carica* (15), *Strophantus regalis* (14), *Strophantus scandens* (14), *Antiaria toxicaria* (14), and of various Euphorbiae (16). He states that a study of the cultures proves conclusively that the flagellates and amoebae belong to the same cycle. It seems to us that in order to prove this point cultures started from a single protozöon would be most desirable. Franchini has found that amoebae from the latex of the fig (15) and a few other hosts, when cultured on blood agar, ingest blood corpuscles (14), behaving like pathogenic amoebae in the intestinal wounds of a patient suffering from amoebean dysentery, and differing therein from trypanosomides, which are known to depend entirely on osmotic exchanges for intake of nutritive material. The author finds that bacterial contamination has an inhibiting effect on all the parasites (14).

Franchini has not yet been able to study the modes of infection and life cycles of any of the many forms which he has discovered. Roaches, plant lice, flies, and even spiders are suspected of being the alternate hosts (11, 14).

Although hasty, this series of preliminary notes by Dr. Franchini opens a very remarkable field to investigation, showing that the presence of protozoa in laticiferous plants is very general indeed.

In order to make this review of literature more nearly complete, mention should be made of a paper by Dr. Franchini (13) in which he reports a case of cabbage flagellosis. In smears made from yellow cabbage leaves on which numerous Hemiptera had been feeding, he finds flagellates similar to those found in the intestines of the insects. From such observations he announces that he has found a flagellosis of cabbage. The evidence, however, is not convincing, as the technique does not entirely preclude the possibility of contamination of the smears from insect excreta present on the leaves.

It is hoped that Dr. Franchini will, in the near future, supplement his notes by a detailed and careful study of the numerous protozoa which he has reported.

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ALTERNARIA SOLANI AS A CAUSE OF TUBER ROT IN POTATOES

DONALD FOLSOM AND REINER BONDE

WITH PLATES V TO VII

In the extensive literature upon the early blight of potato caused by *Alternaria solani* (E. & M.) J. & G., the writers have failed to find any mention of the occurrence of tuber rot attributed to this fungus. In his monograph upon early blight, Rands mentions tuber infection only in relation to a certain hypothesis of interseasonal perpetuation. He states (1, p. 32) that he has "no evidence to substantiate," and sees "no reason for accepting the hypothesis offered by Massee and endorsed by McAlpine that the disease is transmitted from one generation to another by latent mycelium in the tubers." The writers infer that Rands also had no evidence of tuber infection of the rot type. The present paper is submitted as being of interest and importance in regard to the effects of the early blight organism on potatoes.

In March, 1922, a small lot of Spaulding Rose potatoes was received from an experienced grower in central Maine who did not recognize the nature or cause of certain spots found on tubers taken from a storage bin. One of these tubers is depicted in plate VII, fig. A. A visit by the senior writer to the bin in question disclosed considerable bruising and rot, the latter apparently of the Fusarium dry-rot kind, and but very few examples of the spots in question. Enough material, however, was secured to permit a few isolations, nineteen of which gave cultures of a certain type. Of these nineteen similar cultures, some produced spores characteristic of *Alternaria solani* (Plate V, Fig. A), and seventeen of them, when grown on potato agar, caused the typical discoloration of the medium described by Rands (1, p. 20), yellow at first, changing to reddish. Two of the nineteen cultures caused no discoloration, although the mycelium and spores were typical for the species in question.

About a year previous, several tubers with similar spots had been noted during the planting season and planted separately. No cultures were made from these tubers. There were no effects upon the vines. The tuber progeny of the vines showed no spots. Because of this, and because other problems were more pressing at the time, nothing further was done in 1922, except to assure the maintenance of some of the culture strains in stock. In January, 1924, Dr. L. O. Gratz, of the Florida Agricultural

Experiment Station, sent a similar lot of Spaulding Rose potatoes showing various degrees of injury from the small lesions or spots to complete decay. He stated that the trouble seemed to be of considerable importance in seed sent from Maine to Florida. A somewhat similar lot, mostly in an advanced stage of decay, came from the same region in Florida on seed tubers of the same variety, through a visitor from Maine. Isolations by the writers yielded various fungi, including several species of *Fusarium*, the latter being found especially in the more advanced stages of decay, and also yielded a number of cultures of *Alternaria solani.* These cultures produced a few spores even with ordinary methods and, with one exception, discolored the agar.[1] Spores were also obtained in abundance *in vitro* from the mycelium by shredding the agar as suggested by Rands (2). At this time it was possible to attempt inoculations of plants and tubers.

SYMPTOMS IN THE TUBERS

The spots or lesions usually are darker than the healthy skin and appear somewhat sunken, sometimes surrounded by a slightly raised border (Plate VII, Figs. A and B; Plate VI, Figs. C and D). Their outline may be circular or irregular. The lesions are nearly always shallow (Plate VI, Fig. D) and small, rarely exceeding a centimeter in diameter, and the transition from diseased to healthy cortical tissue often is abrupt enough that it is easy to lift out the infected portions intact. Apparently the invasion of new tissue by the parasite often ceases, and is slow when it continues after the first stage (Plate VII, Figs. B and C). When *Alternaria solani* was recovered from large lesions, other fungi were usually associated with it. That these fungi were not the initial cause of the decay is indicated by their miscellaneous nature and by their absence from the small apparently primary lesions.

ARTIFICIAL INOCULATIONS

The first inoculations were made on Green Mountain tubers and plants during March and April, 1924. The mycelium, with the normal scarcity of spores, was rubbed upon the surface of young tubers freshly dug in the greenhouse. This was done at various times. Infection frequently resulted, usually at the lenticels, but also where needle pricks had been made and sometimes at the eyes (Plate V, Fig. B). The fungus was recovered from the resulting lesions and was used to make similar lesions on other tubers. Tuber controls upon which sterilized mycelium was rubbed or which were untreated remained healthy even when in the same damp chamber (Plate V, Fig. B). For further confirmation of identification, the mycelium, both before the inoculation of the tubers and also after being recovered from them, was rubbed upon the surface of the leaves of small but rather mature plants grown in the greenhouse. Early blight had not been observed during the last six winters under these greenhouse conditions where the floors are of concrete, the application of steam heat is sometimes continuous during the day throughout the winter, and the air consequently is apparently quite dry as a rule. Here it was difficult to secure infection in the open greenhouse by inoculation. However, infection with the mycelium was readily obtained under bell jars in which the air was humid (Plate V, Fig. C). Control plants and uninoculated control leaflets and leaves on the same plant remained healthy (Plate V, Fig. C). Infection sometimes was so severe that yellowing, collapse, and death occurred within a few days after the spots appeared. It may be pointed out here that in a similarly humid atmosphere, namely, in Bermuda, similar rapid devastation has been reported by Whetzel (3). With spores obtained *in vitro*, also, no foliage infection was obtained in the open greenhouse, but it was readily secured under bell jars. Here, again, yellowing and collapse often occurred (Plate VI, Figs. A and B). But where the amount of inoculum applied was not excessive, spotting occurred, resembling somewhat in distribution upon the leaves conditions seen in badly infested fields. However, the characteristic target-board zonation was not so apparent, and lesions also occurred on the petioles and stems, again resembling the effects seen in Bermuda (3, p. 101). Spores were readily obtained from the diseased foliage, confirming the identification made *in vitro*.

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[1] Dr. Gratz has reported, orally to the writers, similar results from isolations made in Florida. He also examined the manuscript for this paper before its submittal for publication.

Reference has been made to the occasional discovery of cultures or strains that were non-chromogenic with respect to potato agar. A preliminary comparison was made between chromogenic and non-chromogenic strains in regard to pathogenicity, and, incidentally, between strains of 1922 and those of 1924. Greenhouse plants were inoculated with the mycelium of four strains: chromogenic, isolated in 1922; non-chromogenic, isolated in 1922; chromogenic, isolated in 1924, and non-chromogenic, isolated in 1924. No difference was apparent in the resulting infection of the foliage.

In order to determine the natural method of infection, spores, instead of the mycelium, were applied to young tubers by means of an atomizer. The results were similar to those obtained with the mycelium. Isolations were made from the lesions, and the cultures produced spores of *Alternaria solani*.

FIELD INOCULATIONS

In the late summer of 1924, diseased Irish Cobbler foliage and somewhat immature Spaulding Rose tubers were packed together at various times. Usually abundant lesions resulted (Plate VI, Figs. C and D; Plate VII, Fig. B). When controls consisted of tubers not packed in the leaves, no infection occurred. Sometimes such a control was divided after infection had appeared in the corresponding inoculated tubers, and part of the control was inoculated in the same way, with similar positive results. When well-sprayed, comparatively healthy foliage was packed with the tubers, only a few lesions appeared. All platings made from lesions soon after their appearance yielded pure cultures of *Alternaria solani* (Plate VII, Fig. C).

These results suggest the possibility of infection of freshly dug tubers from contact with diseased foliage. In Maine, digging is necessarily often done before the vines are all dead, or within a few days after the vines have been killed wholly or in part by low temperature. The death of the vines in the former case may be hastened by early blight. Therefore diseased foliage often comes into contact with freshly dug, somewhat immature tubers which are more or less bruised by the time they are put into storage (Plate VII, Fig. A).

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DESCRIPTION OF PLATES

PLATE V

FIG. A. Photomicrograph of spores of *Alternaria solani* as usually secured from cultures in these experiments. X 400.

FIG. B. (I) Young Green Mountain tuber inoculated and infected with the mycelium of *Alternaria solani* cultures isolated from tuber lesions. (C) Control.

FIG. C. (I) Leaf of Green Mountain greenhouse plant inoculated and infected with the mycelium of *Alternaria solani* cultures isolated from tuber lesions. (C) Control.

PLATE VI

FIG. A. (I) Green Mountain plant inoculated and infected with *Alternaria solani* spores obtained *in vitro* from cultures isolated from tuber lesions. (C) Control. Compare with fig. B.

FIG. B. (I) Leaves from the inoculated plant of fig. A. (C) Leaf from the control plant of fig. A. The small spots are caused by thrips.

FIG. C. Infected Spaulding Rose tuber photographed soon after being packed in diseased foliage. Compare with fig. D.

FIG. D. Infected Spaulding Rose tubers cut to show the shallowness of the lesions. Photographed soon after infection resulting from being packed in diseased foliage. Compare with fig. C.

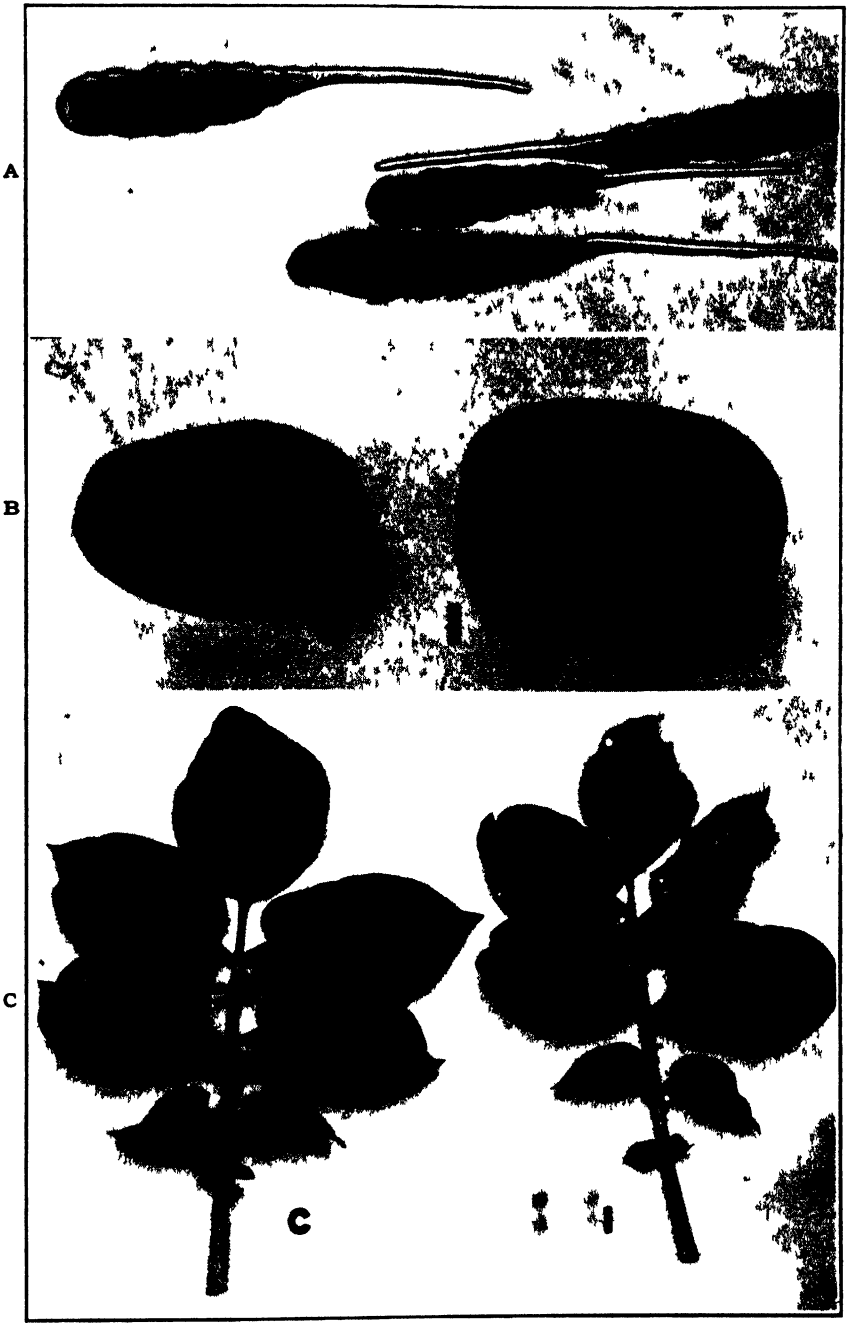
PLATE VII

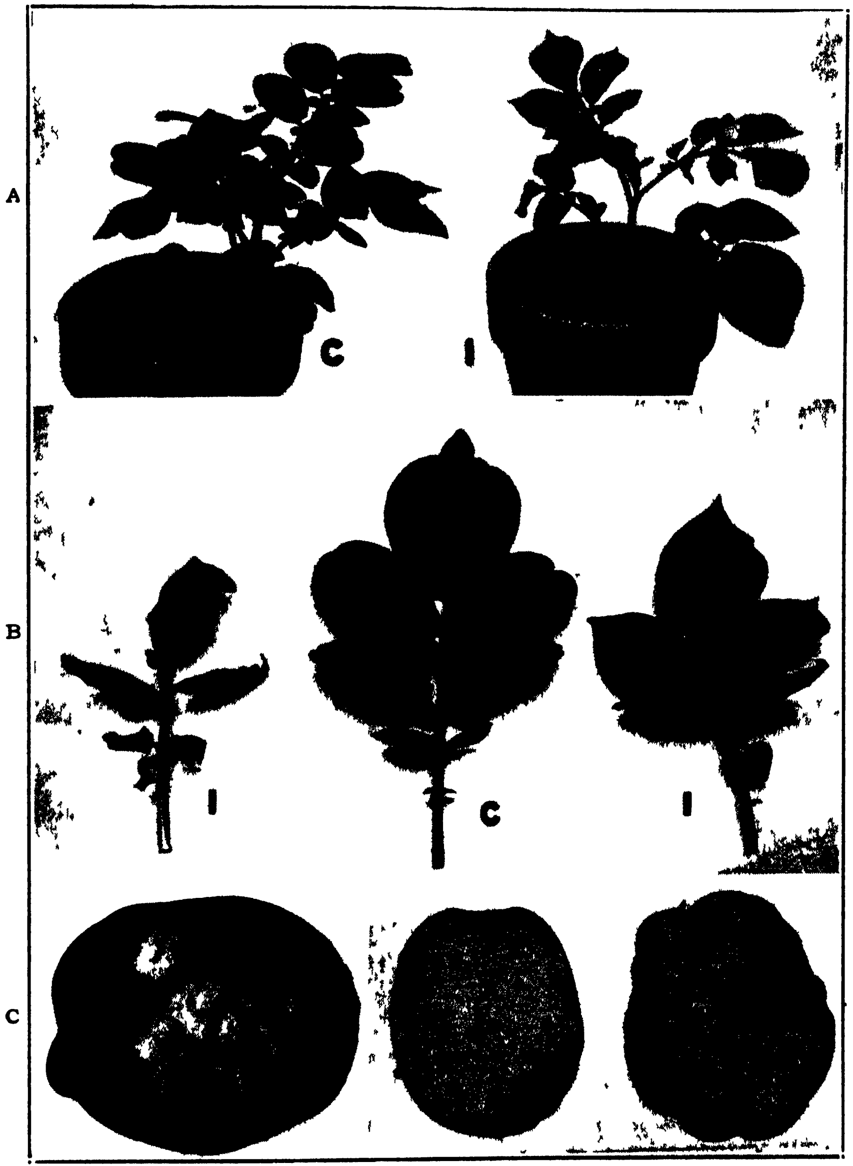
FIG. A. Spaulding Rose potato with early blight lesions occurring where the skin has been partly rubbed off, probably during digging. Photographed in March, 1922, after the tuber had been in cold storage through the winter. Compare with fig. C.

FIG. B. Infected Spaulding Rose tubers photographed soon after being packed in diseased foliage. Compare with fig. C.

FIG. C. The same tubers as of fig. B, after about two months, not in cold storage. The scars were caused in the process of isolating the fungus. Compare with fig. A.

PLATE V

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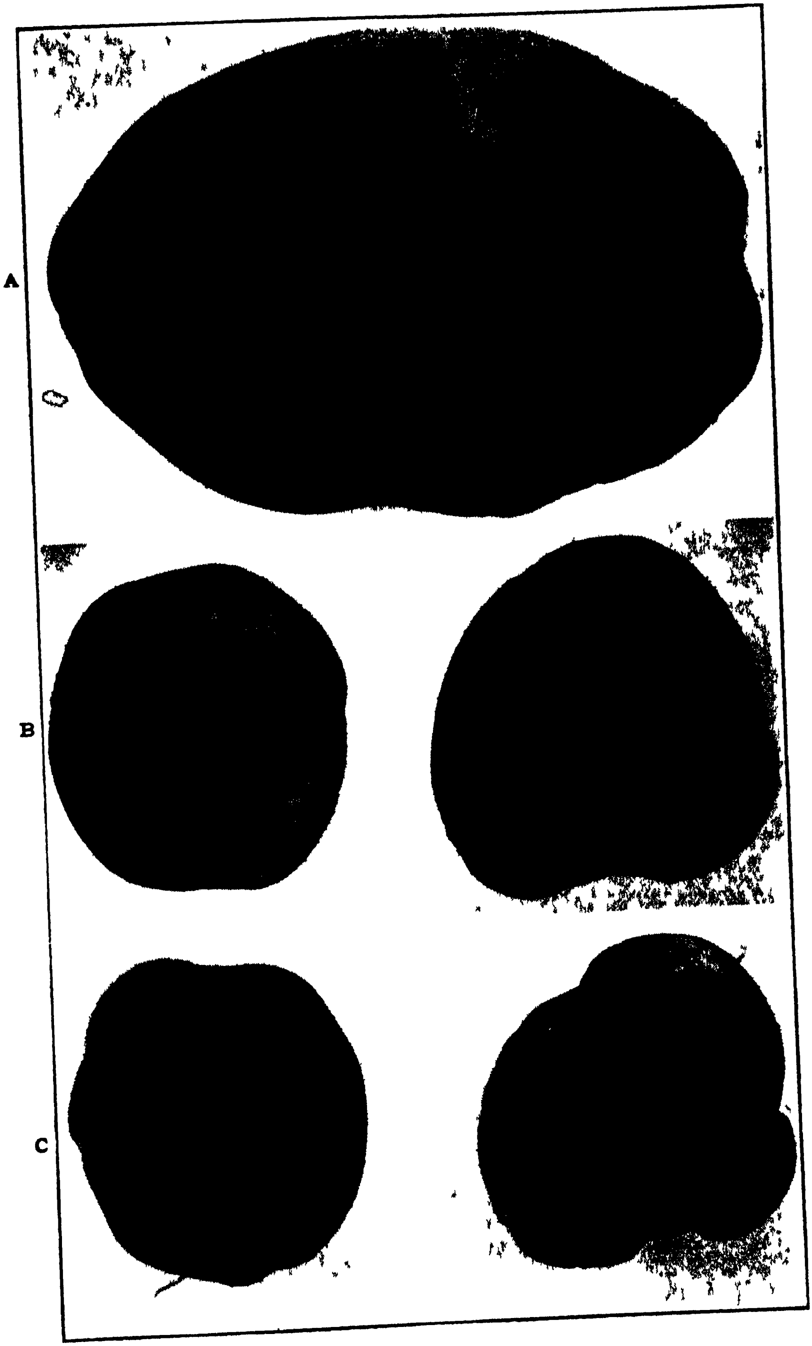
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THE RELATION OF SOIL MOISTURE TO THE POX OR GROUND ROT DISEASE OF SWEET POTATOES

R. F. Poole[1]

With Four Figures in the Text

During the last four years, results of observations on the prevalence of the pox or ground rot disease of sweet potatoes (Figs. 1 and 2) caused by the slime mold, *Cystospora batatas* E. & H., show that it was worse in dry than in wet seasons, notably so when there was drouth in June and July. A very striking demonstration at the Vineland Training School farms on experimental plots showed slight infection in the dry season of 1921, no infection in the wet season of 1922, and severe infection in the very dry season of 1923. In severely infested soils in the Swedesboro locality, the disease causes nearly a total failure of the crop in dry seasons, but on the same soil in wet seasons fair to nearly normal yields are produced. These results suggested that some rather definite information might be obtained by studying the relation of soil moisture to the disease under controlled conditions in the greenhouse.

REVIEW OF LITERATURE

A review of literature on the pox disease showed no definite record of the moisture relations of the disease. As the result of field observations, a number of workers, including Halsted (3), Duggar (2), Adams (1), Poole (4, 5), and others, reported that the disease was worse in dry than in wet seasons. Halsted said, "In a dry season the plant is less able to push ahead and overcome the attack, and therefore the yield is light and the rot appears more abundant. That the season may be favorable will always be a hopeful element in the grower's calculations." Taubenhaus (6) comes to a different conclusion by saying, "In wet weather pox is just as severe, and the causal organism perhaps more active, but the spots are more shallow and less noticeable."

METHODS USED IN CONDUCTING THE EXPERIMENTS

The soil, a sassafras sand, in which 85 per cent poxed sweet potatoes were produced in 1921, was obtained near Mickleton, New Jersey. It was sieved to remove stones and débris and air-dried in the greenhouse all winter. One-gallon glazed pots were used for the Vineless Yellow Jersey

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[1] Paper No. 225 of the Journal Series New Jersey Agricultural Experiment Stations, Department of Plant Pathology.

variety, and two-gallon pots for the Triumph variety, nearly filled with 7,000 and 10,000 grams of well mixed dry soil, respectively. The water-holding capacity of the soil taken from two parts of the same field was determined by use of Hilgard pans and found to be 18 and 20 per cent. When water was added to 7,000 and 10,000 grams of air-dried soil until it was so well saturated that a slight excess of water remained on the top, approximately the same percentage was obtained as with Hilgard's pans. The range of moisture was then prepared on the basis of the water-holding capacity of the soil (Fig. 3).

The soil used in these experiments, being sandy and of a low water-holding capacity, remained loose throughout the experiment. The moisture percentages were maintained satisfactorily by weighing the pots at intervals of from one to three days, depending on weather conditions, and adding water to make up the weight lost. After finding that the water diffused readily and quite evenly in the high moisture pots, and that very little watering was required to keep the low moisture percentages normal, no effort was made to sub-irrigate or retain moisture with surface coverings. Three sprouts were set in each pot in order to obtain a large number of feed roots, "feed roots" meaning those which do not become potatoes or storage roots.

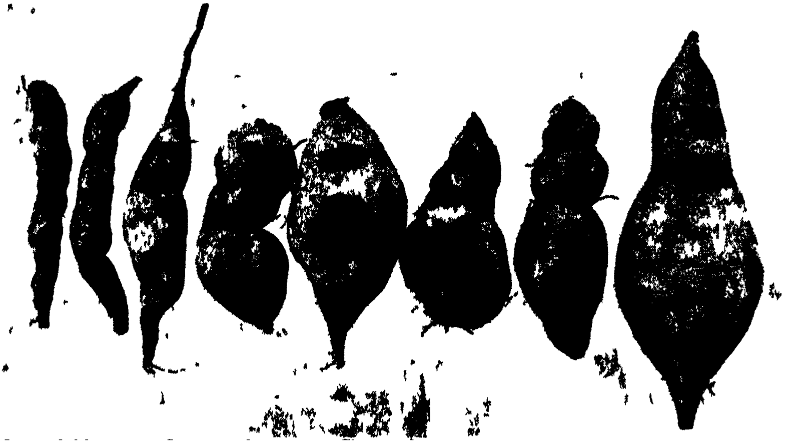

FIG. 1. Pox or ground rot disease of sweet potatoes. Note deep markings of spring and early summer infection, and small shallow spots of late summer and early fall infection.

EXPERIMENTAL RESULTS

Experiment I was a preliminary test to determine if the organism remained active after air-drying the soil. This work was conducted from October 1, 1923, to February 1, 1924, using the Big Stem, Red, Vineless and

Yellow Jersey, Dooley, Nancy Hall, Porto Rico, Triumph, Red Brazil, and White Yam varieties. The roots of all of these varieties were attacked, but the Jersey varieties were most severely attacked. The results showed that the parasite remained very active, and that the Triumph variety, though less severely diseased, produced a very good root system, even in very dry soil. In view of this fact, the Triumph and Vineless Yellow Jersey varieties were selected as being more suitable for pot studies than some of the other varieties.

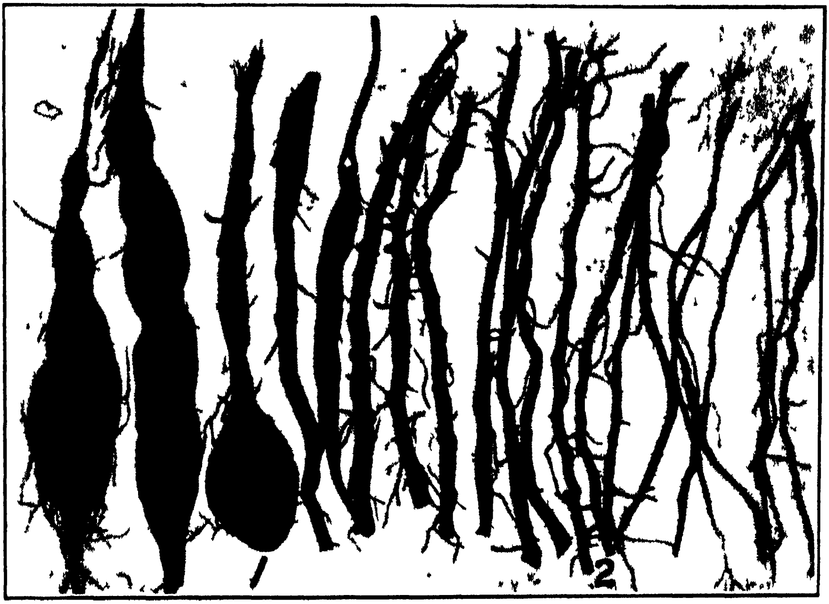

FIG 2 (1) Potatoes severely poxed (2) Roots showing wide variation in infection and size of spots

Experiment II was conducted with the Triumph variety with a 2, 5, 10, 15, and 20 per cent moisture range from April 29 to October 2, 1924, when the plants were harvested. In order to recover as much of the root system as possible, the pots were rolled until roots and soil were emptied together. The roots still attached to the plants were then separated from the soil and 25 roots (not potatoes) were selected from all parts of the three plants in each pot and examined to determine the number of poxed spots. The average results from three plants in each treatment are given in table 1, which shows that there was no infection at saturation and 15 per cent moisture, based on the water-holding capacity. There was slight infection at 10 per cent, and very severe infection at 5 and 2 per cent.

TABLE 1.—*The influence of soil moisture on the development of pox*

Moisture per cent (saturation)	Roots examined No.	Roots poxed No.	Poxed spots total No.
20	25	0	0
15	25	0	0
10	25	5	15
5	25	19	85
2	25	31	121

In order to demonstrate that the soil was infected in the high moisture pots and to determine if a sudden drought favored infection, the moisture in one series of pots was permitted to drop to 2 per cent. After 14 days, the results, given in table 2, show that a change from high to low moisture brings about a favorable condition for infection, which became so severe as nearly to destroy the root system. A larger number of poxed spots were produced on roots of plants grown in 5, 10, and 15 per cent than in 2 per cent moisture. The difference is due to the fact that a greater root area was developed and exposed to infection in the higher moisture pots.

TABLE.—*Influence on pox development of suddenly changing the soil moisture from a high to low percentage*

Moisture per cent	Roots examined No.	Roots poxed No.	Poxed spots total No.
20*	25	25	83
15	25	25	147
10	25	25	254
5	25	25	638
2	25	25	145

* The plants were grown in the original percentages of moisture. From September 18 to October 2, they were all maintained at 2 per cent moisture.

Experiment III was conducted with the Vineless Yellow Jersey. Its short vines made it very convenient for handling in pots, and the strain also was very productive of roots. The object of this experiment was the same as in the previous one, but with less increments in the moisture range and more cultures. The plants were set June 12 and harvested August 25. The average results in table 3 show a gradual increase in infection from high to low moisture content, there being no root infection in saturated soil and very slight infection above 12 per cent. The disease was strikingly severe on roots in 6 and 4 per cent moisture, some being blackened for almost the entire length (Fig. 3), due to coalescing of poxed spots. Many of the roots broke off and completely rotted away, especially after being girdled (Figs. 1 and 2). It was impossible to get a complete count of the total number of poxed spots on roots in pots having a low moisture content, as many of the roots were girdled, broken off, and rotted. The potatoes in the 8, 6, and 4 per cent moistures were severely poxed, while in the higher moisture content no infection was observed (Fig. 4). The maximum root growth and also the greatest total dry weight were obtained between 10 and 16 per cent moisture, indicating that the most favorable moisture condition for producing the sweet potato crop is above that favoring severe pox infection.

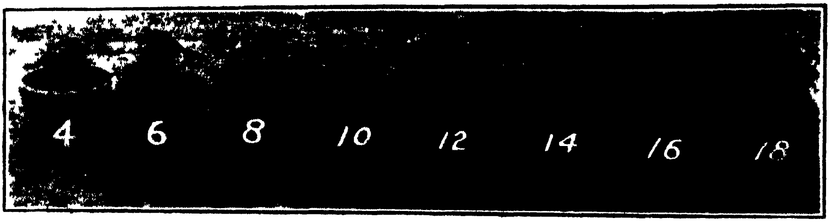

FIG. 3. Moisture range, type of pot used for the Vineless Yellow Jersey variety.

TABLE 3.—*Influence of soil moisture on the development of the Vineless Yellow Jersey Strain*

Moisture (saturation)	Roots poxed No.	Total poxed spots No.	Potatoes raw yield gms.	Vine and roots dry weight gms.
18	0	0	3.4	9.03
16	5	6	31.1	18.74
14	9	12	31.1	20.33
12	16	35	35.1	17.88
10	24	80	25.1	16.14
8	21	84	22.8	14.33
6	24	239	5.2	6.31
4	25	362	.7	2.30

CONCLUSION

The results obtained above suggest irrigation, which might be profitable on the best types of sassafras sand. They also suggest the addition of organic matter to the soil, either through the plowing under of cover crops or the addition of large quantities of organic matter until the soil becomes less susceptible to drought. Furthermore, different methods of culture, especially different depths of planting, should be studied, as well as depths of plowing the land.

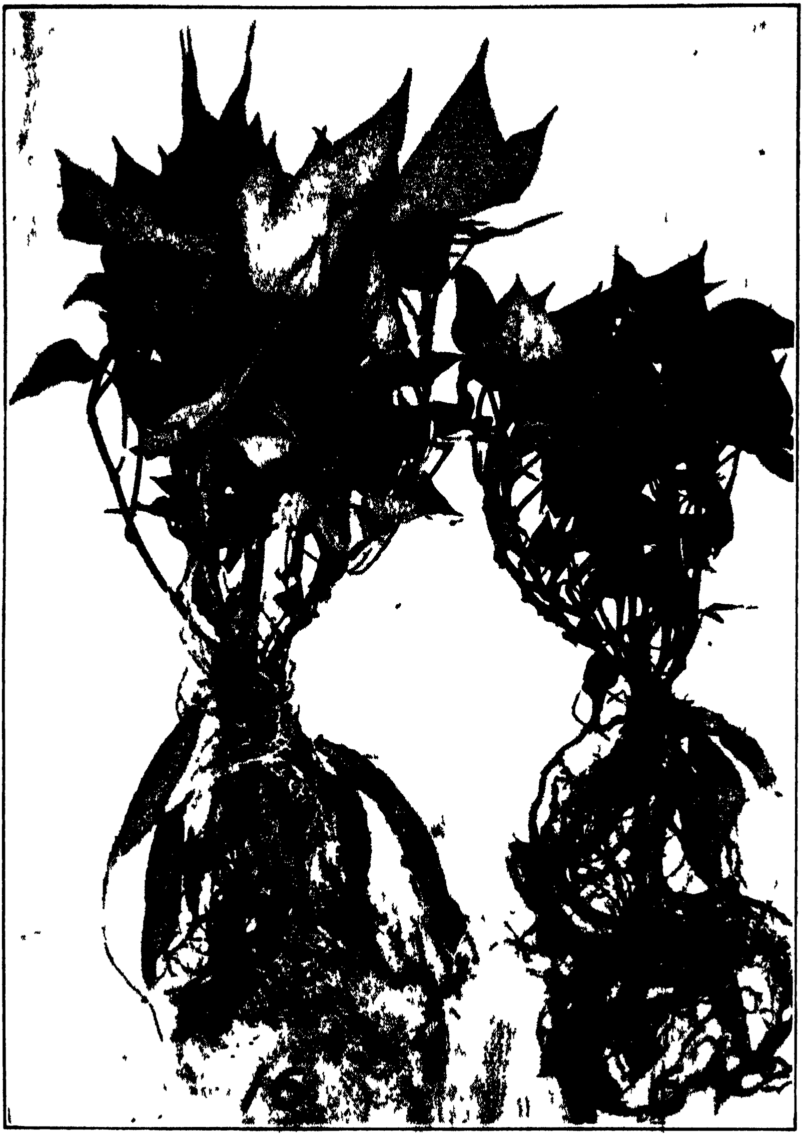

FIG. 4. Plant on the left showing healthy white roots and potatoes grown in 16 per cent moisture. Plant on right showing diseased, severely blackened roots, grown in 6 per cent moisture.

SUMMARY

1. The pox, ground, or soil rot disease, *Cystospora batatas* E. & H., of sweet potatoes, *Ipomoea batatas,* is most severe in dry seasons.

2. The disease attacks the Big Stem, Red, Vineless and Yellow Jersey, Triumph, Dooley, Nancy Hall, and Porto Rico varieties, being most severe on Jersey varieties. The parasite remained very active in spite of the soil being thoroughly air-dried all winter before using it for these tests.

3. The Triumph variety was not attacked at 15 to 20 per cent moisture, the latter being the water-holding capacity of the soil used. It was attacked slightly at 10 per cent and severely at 5 and 2 per cent. Decreasing the moisture to 2 per cent for 14 days resulted in severe infection of all roots that had grown in the high moisture percentages.

4. The Vineless Yellow Jersey showed no infection in saturated soil (18 per cent), and very slight infection above 12 per cent. While there was a high percentage of infection at 8 per cent, it was most serious at 6 and 4 per cent. Maximum production of potatoes as well as of vines and roots was between 10 and 16 per cent, being above the critical point of infection.

5. The results suggest the use of irrigation and the addition of organic matter to make the soil more resistant to drought. They also suggest that different depths of plowing and planting should be studied.

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NON-PATHOGENICITY OF THE MILKWEED FLAGELLATE IN MARYLAND

FRANCIS O. HOLMES[1]

It has been repeatedly affirmed by European authors that the flagellates of the Euphorbias cause pronounced symptoms when they are numerous in the latex of the plants. The leaves become yellow and are easily detached from the twigs, the latex becomes watery and contains little or no starch. Later the latex may even disappear completely.

Observations made in the autumn of 1923 seemed at the time to indicate that Maryland milkweeds (*Asclepias syriaca* L.) showed similar symptoms when herpetomonad flagellates of the species *Herpetomonas elmassiani* (Migone) were present in their latex. At that season the herpetomonads were always very numerous if present at all, and several accidental circumstances seemed to point to the pathogenicity of the flagellates.

It happens that flagellates are found almost entirely in seed-bearing plants. These mature early and are already yellow when non-fruiting plants are still green. Their latex becomes clear and watery as the pods mature, and the leaves drop early leaving the pods alone on the bare stalks. By the time of the actual maturity of the seeds, no latex is to be obtained from the plant. These normal changes late in the season in accidental association with the latex-inhabiting flagellates seemed to parallel European findings as to the starving effect of large numbers of herpetomonads in the latex cells.

It was necessary to study the plants through the spring, summer, and autumn, as was done in 1924, before the effect of the flagellates could be distinguished from the effect of approaching maturity.

EARLY INFECTIONS RARE

Early in the summer a search was made for plants showing flagellates in their latex, but such plants were much rarer in May and June than they were in September and October.

The milkweeds in Maryland pushed up their young shoots from overwintering rootstock buds about the third week of May. A few days later a series of latex smears taken from a hundred scattered plants showed just one to be infected. At this time the plants were not more than a foot and a half high. A week later several additional positive plants were found, not scattered among the various groups examined, but all in the single group in which the first infected shoot had appeared. These few young plants were already heavily infected.

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[1] Joint contribution from the laboratories of the Boyce Thompson Institute for Plant Research and of the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

LACK OF EXTERNAL SYMPTOMS

Throughout the summer these heavily infected individual plants were carefully compared with the more numerous negative shoots of the same group, as well as with negative plants of other groups, until it became evident that no external differences could be found between the plants whose latex contained many flagellates and those whose latex contained none.

Late in the season, as the seed pods approached maturity, the flagellate-containing plants began to lose their leaves, the latex became watery and contained little besides the flagellates themselves. But the plants containing no flagellates followed precisely the same course. The changes were merely autumn modifications. It was impossible after close study to distinguish the infected plants from the others by their external appearance.

França (1) observed that in an infected Euphorbia the presence of *Herpetomonas davidi* in one of two otherwise similar twigs caused a cessation of growth in the infected portion, so that soon the shoot which was free from flagellates had grown far longer than the one in which they were living. The height of the infected milkweeds in Maryland was therefore carefully compared with that of their neighbors which arose from the same root stock but were free from organisms. No difference in height, size of stems or leaves, or numbers of seed pods could be detected. *Herpetomonas elmassiani* seems to be non-pathogenic in the milkweed, *Asclepias syriaca* L.

The case for *Herpetomonas davidi* in Euphorbias is not quite so clear. Lafont (2), França (1), and more recently Strong (5) observed striking changes when the flagellates were present in numbers.

On the other hand, Léger (3), Rodhain and Bequaert (4), and others insisted that it was impossible to predict which plants would prove positive on microscopical examination of the latex and which would prove negative. Perhaps their infections were lighter, or their plants more resistant. It is even conceivable that they were not dealing with the same flagellate species as França's and Lafont's. The specific name "davidi" has been applied to Euphorbia flagellates from countries very widely separated geographically, and sufficient measurements have not been made to determine whether or not the organisms observed by different investigators have been properly named. It may be that some of the flagellates of the Euphorbias, even when present in enormous numbers in the latex, are really non-pathogenic, just as *Herpetomonas elmassiani* in milkweed has shown itself to be.

CONCLUSION

Herpetomonas elmassiani (Migone) may be present in the latex of the milkweed, *Asclepias syriaca* L., in very large numbers without appearing to interfere with the normal growth of the plant or to modify the leaves, stems, or seed pods.

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GEOGRAPHICAL DISTRIBUTION OF THE MILKWEED FLAGELLATE, HERPETOMONAS ELMASSIANI (MIGONE)

FRANCIS O. HOLMES[1]

WITH ONE FIGURE IN THE TEXT

The latex-inhabiting plant flagellate, *Herpetomonas elmassiani* (Migone), was first discovered in the milkweed, *Araujia angustifolia,* in Paraguay in 1916. In 1923 a form morphologically indistinguishable from it was found in the latex of the milkweed *Asclepias syriaca* L. in Maryland. This suggested a possible wide distribution of the flagellate along the Atlantic coast, a matter of some interest as material for class use and for research had not previously been known to exist in the United States. Slides received by the kindness of Dr. R. W. Hegner, of the School of Hygienic and Public Health, Johns Hopkins University, indicate that in the latex of *Asclepias curassavica* L. at Tela, Honduras, the same flagellate abounds. This record from Central America will probably be followed by others from intermediate points along the Atlantic coast of North America and of South America.[2]

Within the United States, the location in Maryland was the only one known in 1923. The following year an attempt was made to find the northern limit of the range of the flagellate. In the early part of the summer infected milkweeds were rare, but in September, 1924, when the organisms were most widespread in Maryland, the survey was started. Among five hundred milkweeds examined in Massachusetts, a few more than two hundred were bearing fruit at the time, but all were negative for *Herpetomonas elmassiani.* The plan of the investigation was to start with this location in New England and to search northward if the location proved positive, but southward if it proved negative, until the boundary of the normal range of the flagellates should be found. As the Massachusetts survey gave negative results, a series from New York State, collected at Yonkers, N. Y., was examined. About the same proportion of fruiting plants was included, one hundred and ninety-nine out of the five hundred bearing seed pods at the time of collection. All were negative.

At the end of September three hundred and fifty plants were examined in Maryland to determine the exact proportion of positive plants there. Seventeen and one-half per cent of *all* the milkweeds collected contained flagellates. Among those which were fruiting a higher percentage obtained, fifty-nine per cent being positive. On the other hand, only five per cent of the non-fruiting plants contained the organisms. On this account, in later surveys only plants in fruit were examined, as they offered by far the most favorable chance for discovering the herpetomonads. The occasional occurrence in non-fruiting plants may have meant that infection took place at blossoming time but that seed pods did not develop, or that the fruiting top of the plant was cut or broken off in midsummer with the resulting growth of non-fruiting side shoots. The insect most closely associated with the positive plants, *Oncopeltus fasciatus* Dallas, seemed to be confined to the blossoms and fruit of the milkweed.

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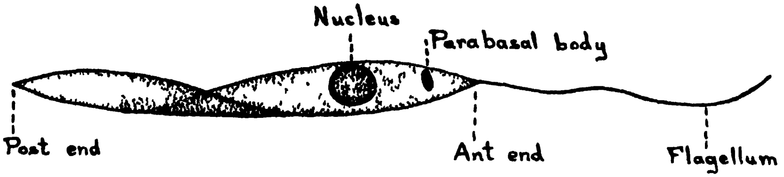
[1] Joint contribution from the laboratories of the Boyce Thompson Institute for Plant Research and of the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

[2] The species has since been found in material collected in Haiti by Dr. L. O. Kunkel of the Boyce Thompson Institute for Plant Research.



A diagrammatic representation of a typical specimen of the latex inhabiting flagellate, *Herpetomonas elmassiani* (Migone), taken from material collected in New Jersey. x 4000.

The next step was to study an intermediate point between Maryland, where the flagellates were common, and New York, where they were not to be found. At Bound Brook, N. J., thirty miles southwest of New York City, and hence about forty-five miles from the negative area examined at Yonkers, N. Y., a survey in early October yielded four per cent of positives. This narrowed the doubtful area to northern New Jersey, and gave a hint that the range of the flagellates might correspond with the normal range of the insect mentioned above, as the latter has been collected within twenty-five miles of New York City, but never has been recorded in New York State.

A location was chosen at Closter, N. J., just across the Hudson River from the negative area at Yonkers, N. Y., and within a few miles of the New York line which defines New Jersey on the east and north. There, on account of the frosts, very few fruiting plants could be found. Yet three plants among the first eighteen collected were positive for the latex-inhabiting *Herpetomonas elmassiani.*

A table of measurements is shown herewith to indicate the morphological identity of the New Jersey flagellate with the species as previously obtained from southern locations. A diagrammatic representation of a typical flagellate is given in the accompanying figure.

On account of repeated examinations of milkweeds on the New York side of the Hudson River and of the absence of the suspected insect carrier,

TABLE 1.—*Average measurements of Herpetomonas elmassiani from New Jersey, compared with measurements of the species as found in southern locations*

	Paraguay Measurements given by França, 1921	*Honduras*	*Maryland*	*New Jersey*
Length of body	12–15μ	13.2μ	12.2–15.6μ	13.1–14.4μ
Anterior end to parabasal body	1.5	1.3	1.2– 1.8	1.3– 1.6
Parabasal body to nucleus	1.5	1.3	1.4– 2.2	1.4– 1.8
Length of nucleus	1.5	1.7	1.5– 2.1	1.6– 2.0
Posterior end to posterior margin of nucleus	7.5–10.5	8.6	7.2–10.7	7.6– 9.8

Oncopeltus, from the negative regions studied, it seems likely that northern New Jersey is the limit of the range of the flagellate along the Atlantic coast.

CONCLUSION

Herpetomonas elmassiani (Migone) previously known to occur in Maryland, was found to be present in the latex of milkweeds (*Asclepias syriaca* L.) as far north on the Atlantic coast as the northern boundary of New Jersey, within a few miles of the Hudson River. Points in New York State and in Massachusetts were examined without positive results.

ABSTRACTS OF PAPERS PRESENTED AT THE SIXTH ANNUAL MEETING OF THE CANADIAN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, OTTAWA, ONTARIO, CANADA, DECEMBER 22–23, 1924

Plant pathology in Canada. B. T. DICKSON.

Presidential address, mainly devoted to a review of the pioneer efforts in the field of plant disease work in Canada. Early publications, (1857 on), investigations, teaching, and legislation were embodied and brief consideration was given to recent developments. The address is appearing in Scientific Agriculture for March, 1925.

Some notes on diseases new to Ontario. J. E. HOWITT.

During the summer of 1924, the following new diseases were found in Ontario: Rose Canker, caused by *Coniothyrium werusdorffiae* Laubert, probably synonymous with *Coniothyrium fuckelii* Sacc.; Twig Canker of Elms, thought to be due to *Gnomonia ulmea* (Schw.) Thm.; Celery Yellows caused by an undescribed species of *Fusarium;* Anthracnose of Lettuce caused by *Marssonia panattoniana* (Berk) Mag.

Root rot or blight of canning peas. R. E. STONE.

The root rot and blight of canning peas is doing a large amount of damage in that part of Ontario east and north of Toronto. During the past season an aggregate of more than six hundred acres of canning peas was completely destroyed. Many other fields show partial loss. The growers report the following varieties as being especially susceptible to the disease: Rogers Winner, Alaska, Perfection, Surprise, Thomas Laxton, Advances, Market Gardener, First Earlies, White Admiral, and Horsford. These are the standard canning varieties.

Test plots have been run on certain selections of peas and there is hope of securing peas of good canning quality that are resistant to this trouble. Horel, a hybrid variety developed in Wisconsin, is quite resistant and certain selections developed in Michigan also show promise. The work will be continued the coming year.

Taxonomic studies of the organism causing black-dot disease of potato. B. T. DICKSON.

Comparing descriptions and specimens it appears that the organism should be known as *Colletotrichum atramentarium.* It is closely similar to *C. maculans* (*V. maculans*), differing only in spore shape. Cultural studies indicate that, while isolations from Quebec, England, France, Pennsylvania, Ohio, and W. Virginia are of the same organism, there is physiological specialization as well as saltation in it. A full account will appear in PHYTOPATHOLOGY and Mycologia.

Winter blight or streak in tomatoes. R. E. STONE.

This disease of tomatoes has proved troublesome in Ontario for a number of years. The disease is associated with an excess of nitrogen in the soil and deficient potash and phosphoric acid.

Good results in the control of this disease in commercial greenhouses have been obtained by increasing phosphoric acid and potash content of fertilizers applied under cultivation.

Oat smut control tests at Macdonald College during 1924. B. T. DICKSON.

Hulless oats were again artificially inoculated with both smuts. Weather conditions were adverse both at seeding and harvest so yield was not considered. The best results were obtained by using nickel dusts. Typical counts are given in the table below.

Results of treating hulless oats with various fungicides for the control of oat smut

Treatment	Culm count of 3 replicas.	Smutted Heads	Clean Heads	% Smut
No control treatment	2972	1752	1220	58.4
Nickel sulphide dust	2623	22	2601	0.8
Nickel carbonate (H. F. & G.)	2458	41	2417	1.66
Nickel hydrate by caustic pptⁿ.	2369	41	2328	1.7
Copper carbonate.	2453	52	2401	2.1
Nickel hydrate by lime pptⁿ.	2995	70	2925	2.5
Presoak and formalin	2458	81	2377	3.3
Uspulum soak	3344	114	3230	3.4
Semesan dust	2322	148	2174	6.0
Semesan soak	3105	198	2907	6.4

Nickel dust at rate of 3 ozs. per bu. of grain.

A mycologist at large. JOHN DEARNESS.

This subject was treated in a popular manner from two points of view; the pursuit of interests in fungi as hobbies with mycophagical and with histological objectives and as an applied science in the hand of the trained plant pathologist. Fungi, as shown by illustrative examples, are as ubiquitous and vary as greatly in shape, size, structure, nutrition, and reproduction as other kinds of plants. Their commonplace and curious uses as food and medicine were related. Persoon's account of his beneficial experience from subsisting on the peasants' coarse bread, raw fungi, and simple water was cited.

Physicianship to plants is almost the youngest of the professions. Beginning with Dr. J. C. Arthur's appointment at the Geneva, N. Y. Experiment Station in 1884, the rise and progress of professional plant pathology was briefly outlined.

The injury to economic plants from the two important causes, insects and fungi, may, for reasons that were enumerated, be estimated as nearly equal. This basis of estimate, supported by other considerations, showed that an annual loss to Canada of $100,000,000 from fungal (including bacterial) plant diseases would be a very conservative estimate. The loss, whatever it may be, can never be wholly averted; nor can an estimate be made of what professional plant pathology is worth to the country now, or will be in the future. Everyone who has, even only superficially, looked into the subject must believe that Canada is only beginning her duty and opportunity in the matter.

An annotated list of the anthracnoses of plants in Canada and the United States was also presented. The list contains fungi of the following genera—*Gloeosporium, Colletotrichum, Marssonia, Septogleum, Cylindrosporium.*

Experiments on sex with mushrooms and toadstools: A report of the work of Irene Mounce, William F. Hanna and Dorothy E. Newton. A. H. REGINALD BULLER.

A description of: (1) the Dry-needle method for making monosporous cultures of Hymenomycetes; and of (2) the Cover-glass Method for obtaining the four spores of a single basidium so that they may be picked up one by one and be sown separately in hanging drops of the culture medium. In the genus *Coprinus* some species, *e.g.*, *Coprinus sterquilinus*, *C. stercorarius*, and *C. narcoticus* are homothallic (Mounce) while other species are heterothallic. Of the heterothallic species: (1) some, *e.g.*, *C. Rostrupianus*, are bisexual and each basidium bears two spores of one sex and two spores of the opposite sex (Newton); while (2) others, *e.g.*, *C. lagopus* (Hanna) and *C. curtus* (Newton) are quadrisexual. In *C. lagopus* some of the basidia bear spores of only two kinds, two of one sex and two of another and opposite sex; while other basidia bear spores of four kinds, each one differing in sexual constitution from the other three. In this fungus, reduction takes place in the basidium during the second division of the fusion nucleus. Two pairs of Mendelian factors are involved in the sexual process. (Lantern Slides).

A study of decay in the balsam fir. A. W. MCCALLUM.

During the summer of 1923, a study of decay in balsam fir was carried out in Quebec. The particular area selected was on the Shipshaw River on the limits of Price Brothers and Company. Here 532 trees were felled upon selected quarter-acre plots so as to secure an average of conditions in the stand, and complete notes were taken for each tree upon all points which might be of value. Two decays of importance were met with: red heart rot due to *Stereum sanguinolentum*; and feather rot, a butt rot probably caused by *Poria subacida*. The characteristics of these decays are described. In balsam fir cut for pulpwood, no other cause of cull but decay occurs. A direct relation was found to exist between age and decay. Starting from zero in the 51–60 year age class the amount of cull steadily increases until in the age class 181–190 it amounts to 40% of the merchantable volume. Although there is a general impression to the contrary, no relation of importance was found to exist between the present condition of balsam fir in regard to decay and the recent budworm outbreak.

A bacterial disease of tomatoes new to British Columbia. H. R. MCLARITY and T. M. C. TAYLOR.

A bacterial disease of tomato is reported as appearing for the first time in British Columbia. Its extent is considered very limited, but in the worst affected plot approximately 75 per cent of the crop was lost. A sudden wilting of leaf or leaflet without change of color is the first evidence of the disease. Shrivelling, browning, and defoliation of the affected tissue, and the appearance of small, sunken, brown areas and some longitudinal cracks on the leaf petiole or stem are later characteristics. The causal organism was isolated and some ninety-two successful inoculations were made on healthy plants. Cultural and morphological characteristics were studied, and it is considered by the writers that the pathogene is identical with that described by Dr. Erwin F. Smith as *Aplanobacter michiganense*. Details of the experiment are to be found in the annual "Report of the Dominion Botanist, 1924."

Oat smut infection in relation to size of grain. J. G. COULSON and E. A. LODS.

Preliminary experiments, carried on at Macdonald College during 1924, to ascertain the relationship between size of kernel and resulting infection, when using oats artificially inoculated with *Ustilago avenae* (Pers.) Jens. and *Ustilago levis* (K. & S.) Mag., indicate, without exception, that a greater percentage of infection results by using small kernels as compared with large kernels. In order to make this evident with Banner and Alaska oats the seeds were de-hulled before inoculation. That de-hulling is necessary is shown in the table in the case of Banner. While the germination of the small kernels was not quite so good as that of the large kernels, the difference was not sufficiently marked to modify the results in the table below.

Experiments to test relations of size of oat seed to infection with smut.
Summary of 1924 results

A. Field tests with Ustilago levis (K. & S.) Mag.—five replications for each test

Variety	Condition of grains	Weight per 100 seeds in grams		Per cent infection by plants	
		Large	Small	Large	Small
Avena sativa var. Banner (4707)	De-hulled	2.94	1.44	19.11 ± .5	35.46 ± 2.02
Avena sativa var. Banner (4707)	Hulled	3.97	1.66	2.01 ± .42	1.75 ± .57
Avena sativa var. Alaska (412)	De-hulled	2.99	1.40	10.95 ± .809	40.73 ± 1.42
Avena nuda var. Liberty (0–480)	Hull-less	3.17	1.41	40.84 ± 3.397	66.14 ± 1.478

B. Greenhouse tests with Ustilago avena (Pers.) Jens.

Avena nuda var. Liberty (0–480)	Hull-less	....	....	32.1	54.84
Avena nuda var. Liberty (0–480)	Hull-less	....	....	2.00	10.10
Avena nuda var. Liberty (0–480)	Hull-less	....	....	36.23	46.70

REPORT OF THE SIXTEENTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The sixteenth annual meeting of the society was held at Central High School, with headquarters at the New Ebbitt Hotel, Washington, D. C., December 29, 1924, to January 1, 1925. The attraction and convenience of the nation's capital as a meeting place, the fact that many members are permanently headquartered there, and the increased growth of the Society during recent years resulted in a record attendance. An accurate count of the number of members present could not be made, but it is estimated that from 200 to 225 persons, practically all of them members, attended the various meetings.

Fewer papers than usual were presented, probably owing to the fact that titles and abstracts were due November 1, which was two months before the meeting and one month earlier than in other years. Forty-four papers were presented at the regular sessions of the Society and an additional five were contributed to the joint session with the Mycological Section of the Botanical Society of America, making a total of 49 papers as compared with 114 in 1923, 79 in 1922, and 97 in 1921. The abstracts of these papers were distributed at the meetings and later published in PHYTOPATHOLOGY (Phytopath. **15**: 44–60. Jan., 1925). A popular summary of the facts brought out in the papers has appeared in connection with the report of the Permanent Secretary of the American Association for the Advancement of Science (*Science* **61**: 266–267. March 6, 1925).

At the usual joint session with Section G of the American Association, which was held Tuesday afternoon, December 30, four papers were presented as follows: The Origin of the Cycads, by J. C. Chamberlain, retiring vice-president of Section G; Root Studies, by J. E. Weaver; Soil Nutrients, by E. J. Kraus; and Mosaic and Related Diseases, by L. O. Kunkel.

The extension pathologists held an afternoon session at which ways of bringing plant disease control methods into common use were discussed informally, and the subjects of seed treatment and orchard spray service were considered in some detail. The conference was attended by about 55 persons and considerable interest was shown in the program and discussion.

On Wednesday morning, December 31, many of the members accepted the invitation of the pathologists in the Bureau of Plant Industry to visit them in their offices and laboratories, and about 100 persons took advantage of the opportunity to visit the Arlington Farms in Virginia. Three simultaneous sessions, one on vegetable diseases, one on cereal diseases, and one on fruit and miscellaneous diseases were scheduled for Thursday morning, January 1. These sessions were all well attended and active discussion took place at each.

The closing event of the meeting was the Phytopathologists' dinner and entertainment at the New Ebbitt Hotel the evening of January 1. At this meal, 316 persons were served, this being 160 more than at Cincinnati in 1923, and 200 more than at Boston in 1922. The entertainment consisted for the most part in a minstrel show staged by some of the Washington members.

OFFICERS AND REPRESENTATIVES

The following officers were chosen, the first four elected by the Society and the others selected by the Council and approved by the Society.

President, C. W. Edgerton, Agricultural Experiment Station, Baton Rouge, La.

Vice-president, M. F. Barrus, N. Y. State College of Agriculture, Ithaca, N. Y.

Councilor (two years) Wm. Crocker, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.

Representative on Board of Control of Botanical Abstracts (four years), G. R. Lyman, University of West Virginia, Morgantown, W. Va.

Councilors, representing Divisions of the Society, W. T. MacClement, Queen's University, Kingston, Ontario, Canada, representing the Canadian Division; W. T. Horne, University of California, representing the Pacific Division; C. A. Ludwig, Agricultural Experiment Station, Clemson College, S. Car., representing the Southern Division.

Members of the Editorial Board of Phytopathology (chosen by the Council), *Editor-in-chief* (three years, E. C. Stakman, University of Minnesota, St. Paul, Minn. *Editors* (three years), J. G. Leach, University of Minnesota, St. Paul, Minn; L. R. Hesler, University of Tennessee, Knoxville, Tenn. *Associate Editors* (three years), D. L. Bailey, Manitoba Agricultural College, Winnipeg, Manitoba, Canada; H. W. Anderson, University of Illinois, Urbana, Ill.; B. B. Higgins, Agricultural Experiment Station, Experiment, Ga.; W. H. Tisdale, U. S. Department of Agriculture, Washington, D. C.; S. M. Zeller, Oregon Agricultural College, Corvallis, Ore.; B. T. Dickson, Macdonald College, Quebec, Canada; A. W. Henry, University of Minnesota, St. Paul, Minn.; C. A. Ludwig, Agricultural Experiment Station, Clemson College, S. Car.; L. W. Durrell, Colorado Agricultural College, Fort Collins, Colo.

Business Manager (one year), R. J. Haskell, U. S. Department of Agriculture, Washington, D. C.

Advertising Manager (one year), J. F. Adams, Agricultural Experiment Station, Newark, Del.

Representatives on the Council of the American Association for the Advancement of Science (one year),G. P. Clinton, Agricultural Experiment Station, New Haven, Conn.; and W. A. Orton, Tropical Plant Research Foundation, Washington, D. C.

Representatives on the Council of the Union of Biological Societies (one year), C. L. Shear, U. S. Department of Agriculture, Washington, D. C., and E. C. Stakman, University of Minnesota, St. Paul, Minn.

Members of the Advisory Board (3 years), J. E. Howitt, Ontario Agricultural College, Guelph, Ontario, to replace J. H. Faull; F. C. Meier, U. S. Department of Agriculture, Washington, D. C., to succeed R. J. Haskell; and M. F. Barrus, N. Y. State College of Agriculture, Ithaca, N. Y., to succeed himself.

Member Board of Governors of the Crop Protection Institute (three years), selected by the Advisory Board and approved by the Council and Society, N. J. Giddings, University of West Virginia, Morgantown, W. Va. The other two representatives are C. R. Orton and M. F. Barrus.

The following temporary committees which served throughout the meeting were appointed by the President: *Resolutions Committee*, N. J. Giddings, P. J. Anderson, and J. F. Adams; *Auditing Committee*, G. L. Peltier and Chas. Chupp; *Elections Committee*, C. S. Reddy and C. A. Ludwig.

REPORT OF THE SECRETARY-TREASURER, 1924

At the Cincinnati meeting, 88 new members were elected, which brought the total membership up to 645. However, during the past year 31 members were suspended for non-payment of dues, 6 resigned and 2 died, making a loss of 39, bringing the total membership in good standing December 20, 1924, down to 606. Of the 606 members, 102 were life sustaining and 504 regular members. Ten life members were paid up in full December 20, 1924. At the Washington meeting, 59 new members were elected making the present total membership 665.

The accompanying chart (Fig. 1) shows the annual growth of the Society since its beginning in 1910, when it started with 130 charter members. It will be seen that there has been a steady growth in membership with noticeable increases in 1920 and in 1924 and 1925. The figures in parentheses indicate the number of life sustaining members each year. The secretary has been unable to determine the exact number of members in 1911.

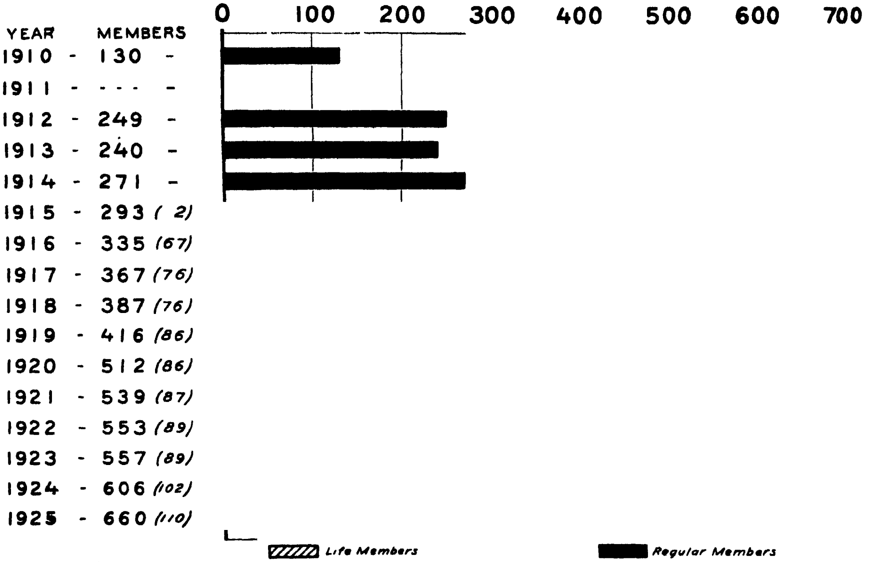


FIG. 1. Number of members, American Phytopathological Society.

Statement of Accounts for 1924, as of December 20, 1924

Receipts:		
Balance from 1923	$1,501.84	
Annual dues	3,256.55	
Cash returned by Secretary-Treasurer on trip to Cincinnati	16.52	
Sales received with annual dues	20.50	
Excess dues	10.90	
Interest on checking account	27.70	
		$4,834.01
Expenditures:		
Amount transferred to sinking fund for investment	$ 490.00	
Secretarial work	209.25	
Postage stamps	15.92	
Account books, telegrams	3.10	
Excess dues returned	3.00	
Secretary-Treasurer's travel and miscellaneous expenses	100.00	
Expenses of Committee on International Botanical Congress	109.82	
Expenses of Program Committee for 1924 meeting	34.32	
Expenses of President to Pan-Pacific Food Conservation Conference	200.00	
Subscriptions for 1923 donated to European countries	44.02	
Sales transferred to PHYTOPATHOLOGY (received with dues)	20.00	
Subscription to Oberly Memorial Fund	3.16	
Stationery, stamped envelopes, printing	96.88	
		$1,329.47

Balance			$3,504.54
Outstanding check			34.32
			$3,538.86
Time deposit of $500 and interest			540.20
			$4,079.06
Amount of above receipts credited on 1925 and 1926		$2,004.51	
Sinking fund:			
Amount due for 1922	$ 5.00		
Amount due for 1923	5.00		
Amount due for 1924	35.00	45.00	
Amount due PHYTOPATHOLOGY for Vol. XIV to European Pathologists		40.00	
Amount due PHYTOPATHOLOGY for member subscriptions		2,000.00	
			$4,089.51
Deficit for 1924			$10.45

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1924

The closing year has been the most prosperous in the history of PHYTOPATHOLOGY. The balance of $3,105.95 reported herewith is the largest the journal has ever had. The receipts have exceeded those of any other year and the amount of printed matter put out has equalled that of 1918 when the record number of pages were published.

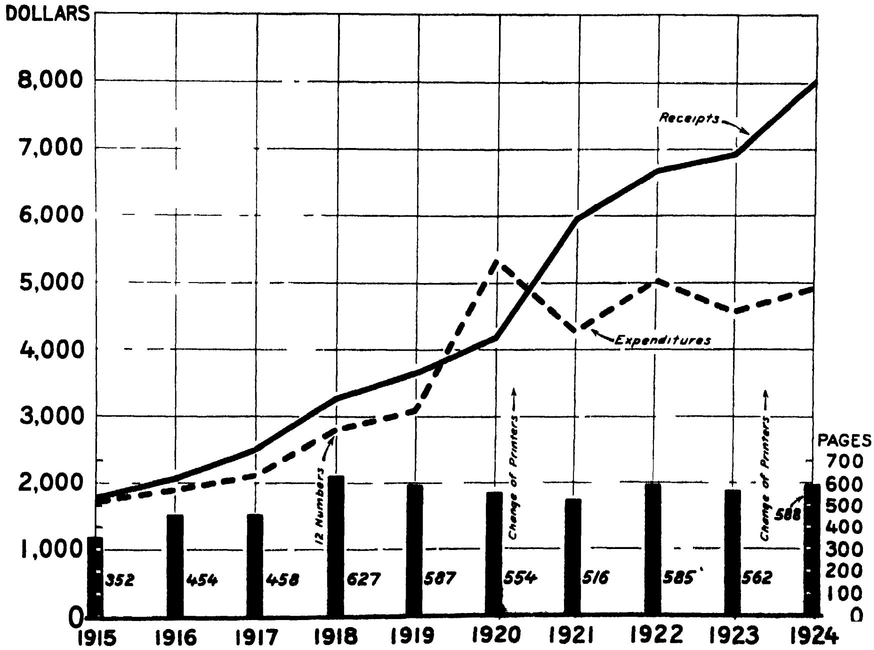


FIG. 2. Receipts and expenditures of PHYTOPATHOLOGY, 1915–1924

The accompanying graph (Fig. 2) shows the receipts and expenditures for PHYTOPATHOLOGY over the ten-year period 1915–1924. The figures used as a basis for the chart were taken from the various annual reports of the business manager as printed in PHYTOPATHOLOGY, and are the same as the totals given there, except that certain additions or subtractions have been necessary on account of items relating to loans, sinking fund, etc., which often appear in both the receipts and expenditure columns. The graph shows a steady increase of income from year to year and also a general increase in expenditure. From 1915 to 1919 the receipts kept pace with expenditures, but during 1920 printing costs mounted so rapidly that in that year a deficit was incurred, it costing $5,329.00 to print 554 pages. To meet the emergency there was borrowed from the treasury of the Society $1,375, and at the Chicago Meeting $1,059.72 was subscribed by the membership. A change of printers was negotiated and the business management took over many of the services for which the publishers were formerly paid. These services included the handling of all subscriptions and advertisements, the sale of back numbers, and the care of mailing lists. The beneficial result of these changes is very evident from the diagram. Since 1920 the journal has not only met its obligations but has been steadily accumulating a surplus until this year it has amounted to slightly over $3,000.

Statement of Accounts for 1924, as of Dec. 20, 1924

Receipts:				
Balance from 1923			$2,270.37	
Subscriptions			2,381.11	
Sales			400.60	
Advertising, 1923			89.80	
Advertising, 1924			607.49	
Interest on invested sinking fund			232.48	
Balance due sinking fund to March 7, 1924			490 00	
Subscriptions for Europeans for 1923			44 02	
Principal of Phelps mortgage note paid up			500 00	
				$7,015 87
Expenditures:				
Manufacturing PHYTOPATHOLOGY:				
Vol. XIII, No. 11	300.51			
Vol. XIII, No. 12	344.58			
Vol. XIII, Index	153.50			
		$798.59		
Vol. XIV, No. 1	405.30			
Vol. XIV, No. 2	384.25			
Vol. XIV, No. 3	352.30			
Vol. XIV, No. 4	312.80			
Vol. XIV, No. 5	278.25			
Vol. XIV, No. 6	248.36			
Vol. XIV, No. 7	374.00			
Vol. XIV, No. 8	312.43			
Vol. XIV, No. 9	218.56			
Vol. XIV, No. 10	264.52			
Vol. XIV, No. 11	311.65			
Engravings for Vol. XIV	338.78			
		$3,801.20		
			$4,599.79	

Miscellaneous expenses, (Dix lists)	56.75	
Second class postage on PHYTOPATHOLOGY	12.03	
Secretarial work	54.00	
Printing Phytopathological Abstracts	93.30	
Postage	19.00	
Expressage, freight, packing, etc.	46.93	
Expenses of Business Manager to Lancaster, Pa.	14.56	
Postage on back volumes shipped by Dr. C. E. Temple	8.77	
Sinking fund invested with accrued interest	1,003.79	
Miscellaneous expenses, account books	1.00	
		$5,909.92
Balance		$1,105.95
Amount of 1925 subscriptions included in above receipts		599.09
		$ 506.86
Amount due PHYTOPATHOLOGY from Society for member subscriptions		2,000.00
Actual balance for 1924		$2,506.86

Members may be interested in noting the various sources of income to PHYTOPATHOLOGY and the relative amounts of each extending over a period of years. With this in mind Fig. 3 has been prepared. It will be seen that the greatest source of revenue is the subscriptions from institutions and non-members. Next in importance come the subscriptions from members. This is followed by receipts from advertising which have been increasing steadily during the past four years, and finally by sales of back numbers of the Journal. The income from all of these sources increased greatly after the change of management in 1921.

During the year 1924, a second change of printers has taken place and PHYTOPATHOLOGY is now being printed by the Science Press Printing Company of Lancaster, Pennsylvania. In December, 1923, a bid was received from the Science Press quoting rates considerably lower than those we were then paying. This offer was taken up with the Council at the Cincinnati Meeting, and also by correspondence during the early part of 1924, with the final result that the change of printers was authorized. It was estimated at that time that the saving which could be effected by the change might amount to about $800 per year.

The change was made commencing with Volume 14, Number 5, and at the same time two decided improvements were instituted. In the first place, a higher grade of paper was substituted for that used formerly. This paper makes the text figures much better and is very satisfactory for plates. With the cheaper paper it was formerly necessary to print the plates on separate paper and insert them by hand, an expensive process for which we paid $10 for one plate, $17 for two plates, $24 for three plates, etc. By putting the plates on the same stock that is used throughout the Journal, they can be printed for $1.95 each, which alone represents a saving that offsets the increased cost of the higher grade paper, and, in addition, we have better text figures and a neater appearing journal in all respects. In the seven numbers printed by the new printers, from May to November inclusive, there have been twenty plates for which we have paid $39. With the other paper we would have paid from $150 to $200.

The other important improvement is an increase in the size of the type page, which was increased from 4½ x 7 to 4 2/3 x 7½ inches, resulting in the obtaining of 3½ square inches more printed matter per page. The new printers have put out 377 of these larger pages which represent an increase of about 40 pages in actual reading matter and for which we would have paid about $150 if put on the small sized page.

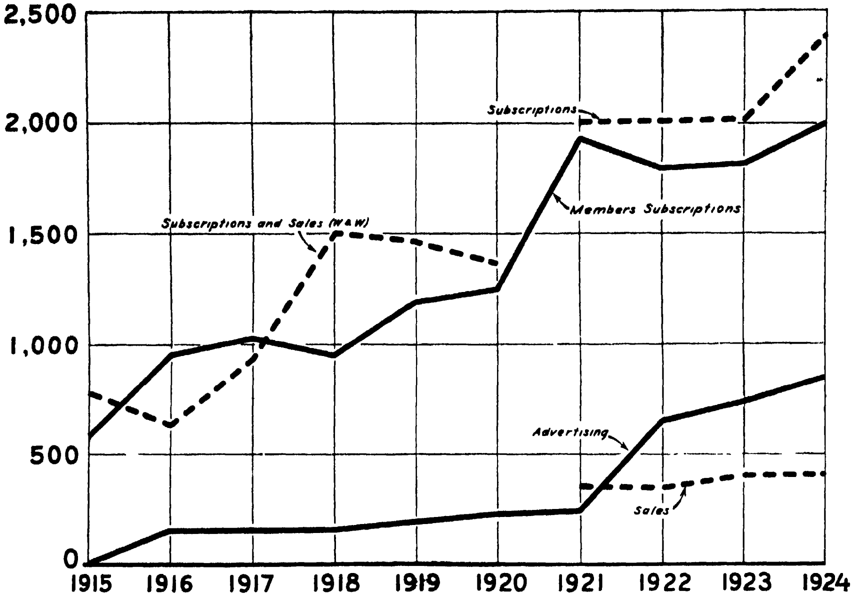


FIG. 3. Receipts from subscriptions, sales of back numbers, members subscriptions, and advertising, 1915–1924

Other economies that have resulted from the change have been figured from the bills for the seven numbers, May to November, when compared with bills from the old printers for the same months during 1923. This comparison shows the following savings:

Mailing list corrections	$ 26.74
Authors alterations	54.70
Discount of 2% for paying bills within 10 days	43.95
Saving on covers	58.80
Total	$ 184.19

To this may be added $135 for saving in cost of printing plates and $150 for the 40 pages of additional printed matter, making a total saving of $469.19 on the seven numbers or a corresponding saving of $804 on twelve numbers.

REPORT OF THE EDITOR-IN-CHIEF OF PHYTOPATHOLOGY

One year ago I resigned as Editor-in-Chief of PHYTOPATHOLOGY. Evidently, like the premature report of the death of Mark Twain, it was not considered a matter to be taken seriously. That resignation was made advisedly, as the conditions which made it necessary have caused undue delay in the publishing of papers in our Journal. This situation is bound to become increasingly worse. Owing to the uncertainty as to when a new Editor-in-Chief would take over the duties of the office, a number of matters have been allowed to drift along which should have been decided before this time. A year ago I gave a resumé of editorial events for the preceding three years. It remains to bring things up to date. During the year 1924, the fourteenth volume of PHYTOPATHOLOGY has been published. It contains 588 pages of text, 106 figures, and 31 plates. During the year we have begun using a calendared paper which is well adapted to use for plates throughout the Journal. This does away with the need to glue in plates, which has been done when a different paper is used for plates, as was formerly done. Although the paper costs more than the old paper, its use actually costs less than was the case formerly, because of this saving of hand work. It is now possible to make text figures of just as good quality as the plates were formerly. Thus illustrations can be placed in the text where they can be most effectively referred to by the reader.

A year ago attention was called to the increasing patronage of our Journal by foreign contributors. Since that time the Society has definitely committed itself to the assumption of an international status by agreeing to publish papers within our field which are written in English, French, or German. An Editor for Europe, Dr. H. M. Quanjer, Wageningen, Holland, has been appointed, and the first papers published under this agreement appeared in the November number. Beginning with the new year, the international status of the Journal will be indicated on the front cover. These are the public editorial features of our entry into the field of international science. The duties of the Editor-in-Chief have been made heavier and more complicated by this innovation. He must be, even more than in the past, in fact as well as in name, Editor-in-Chief.

Finally, I wish to call attention to a matter or rather group of matters which it seems to me will ultimately be of the greatest importance to this Society. Its decision I believe is vital. It is what I choose to call "ethics." It has assumed so much importance with the foresters that they have a subdivision for it in their literature and it is receiving earnest and sober thought by them. It has not appeared as early with us, possibly because we have less direct connections with commercial interests, but it has reached the point where we can no longer ignore it. It is appearing more especially in connection with commercial interests, but there are other angles to the problem which tend toward undermining the very spirit of scientific research, which means unbiased investigations if it means anything. No one who has followed American literature in plant pathology during the last few years (or indeed the literature in any other science) can have failed to notice the increasing disregard for the published work of others. In certain lines of work there seems to be a concerted ignoring of papers published before 1910. While this is true of American literature, it is even more true of foreign literature, whether it be in English or in other languages. I believe every one of us knows workers who do this deliberately. It is one of the most alarming symptoms of the general demoralization which has followed the war. A scientific investigator who ignores antecedently published work connected with his problem lays himself open at best to a charge of carelessness, and at worst to a charge of dishonesty. When a statement is published unsupported by citations, the reader naturally and necessarily assumes that the statement is original, whether the author expressly says so or not. My reason for touching upon this matter in an editorial report is my conviction that this tendency is reaching the point where editorial censorship is quite as necessary as it is for poor grammar or disorderly illustrations. Another reason is my further conviction that editorial policies tending to restrict citation and bibliographies have, in some measure, been responsible for this tendency to ignore previous literature.

Many inexperienced workers feel that if they can not publish their bibliography, or can publish only restricted portions of it, there is no use in looking up the literature at all. While this, of course, is a superficial and absolutely wrong attitude, we should take great care that editorial policy does not become in any way responsible for intellectual dishonesty.

Another angle of the ethics question is what may be called commercialized science. How and to what extent should commercially interested organizations subsidize investigations of their products by public officers? Shall we, by accepting advertisements, assume moral responsibility for statements by the same firm in another publication, which will be placed upon us as surely as we publish the ads? Under what conditions and from what sources can we safely accept financial assistance in publishing our Journal? These all are calling for sober and careful consideration, with a full comprehension of their import before an answer is given. They all have a direct and more or less constant connection with the editorial office.—PERLEY SPAULDING.

REPORT OF THE ADVISORY BOARD

The Advisory Board made the following report to the Council through its Chairman, M. F. Barrus.

The following report consists of a summary of the activities of the committee of the Society and of activities of organizations upon which the members of the Board serve as representatives of the Society.

Summer Meetings. As the British Association for the Advancement of Science met at Toronto during August, no general summer meeting of the Society was held this year. The Pacific Division of the American Phytopathological Society held their annual meeting at Penticton, B. C., on August 26 to 29 in connection with the seventh annual meeting of the Northwest Association of Horticulturists, Entomologists, and Plant Pathologists. This meeting, as reported by Prof. C. W. Hungerford, was a decided success both as to the number in attendance and to the very interesting sessions that were held. There were other meetings of groups of pathologists, but no report of them has been sent to the Advisory Board and those persons in charge of such meetings are not known by members of the Board. It is regrettable that organized meetings of plant pathologists in this country and Canada should take place without there being a general notice of them and an account of their transactions being reported to the Society. An effort is being made by the Advisory Board to provide for a more extended notice of such meetings in the future.

The Advisory Board has accepted, on behalf of the Society, the invitation of the Pacific Division of the American Phytopathological Society to hold its next summer meeting at Corvallis in conjunction with the annual summer conference of that Division. Prof. C. W. Hungerford will represent the Society on the committee of arrangements.

There is unquestionably a long felt need for another cereal-disease conference. As such a conference would probably be deferred during 1926, it seems advisable to arrange for one in some midwestern state during 1925. The Board is unable at the present time to announce more details. It is expected that these two summer meetings will be held at such times as to make it possible for visitors present at the cereal conference to attend the western meeting also.

The attention of members of the Society will be called in due time to a meeting of the Canadian Division at Quebec some time during the summer. It is not unlikely that some members unable to attend the western meetings will be attracted to the Quebec meeting. Details in regard to this meeting will be published later.

The Advisory Board recommends that the summer meeting for 1926 be held in conjunction with the meeting of the International Congress of Plant Sciences to be held at Ithaca, August 16 to 23 of that year. It has been suggested that not only should invitations to attend the pathological meeting be extended to foreign pathologists at various institutions and experiment stations but that they also be invited through the medium of all phytopathological societies in the world.

Phytopathological Institute. The Chairman, Dr. E. C. Stakman, appointed to this project, reports that no action has been taken by his committee in furtherance of the organization of such an institute, and, inasmuch as its function is now being performed by the recently founded Boyce Thompson Institute for Plant Research, he has requested that the committee on Phytopathological Institute be dismissed.

Tropical Research Foundation. The Tropical Research Foundation is an organization formed under the auspices of the National Research Council and incorporated on June 6, 1924, to promote research for advancing knowledge of plants of the tropics by conducting investigations in plant pathology, entomology, plant breeding, botany, forestry, horticulture, and agronomy, and to publish the results of these. In order to accomplish these results, it may establish and maintain such temporary and permanent stations as may be necessary. The American Phytopathological Society is represented by one member, Dr. L. R. Jones, on its Board of Trustees. It is the duty of the Advisory Board in respect to this Foundation to function as a committee on cooperation. Thus far, the only duty the Board has performed has been to endeavor to aid the Scientific Director in the selection of an assistant pathologist. Doubtless in time the Board will be able to render more valuable assistance and should serve as the medium for presenting to the Society the work of the Foundation which has now only just started.

National Research Council. The Advisory Board Chairman of last year, Dr. C. R. Orton, attended two meetings of the Division of Biology and Agriculture of the National Research Council and made a report of the Board's activities to the Division. The Division voted to continue its support of the Arthur rust project for another year. The Society is now represented on the Division only by the Chairman of the Advisory Board who holds his appointment on the Division until July 1 of the year following his retirement as Chairman of the Board.

Promotion of International Relations in Phytopathology. No definite project was undertaken by the Board in this respect. Efforts were made, as in the past, by pathologists at institutions visited during the summer by foreign scientists to entertain them and to assist them in every possible way. Among such visitors were Dr. Wilfrid Robinson, of the University of Manchester, England; Dr. V. H. Blackman, of the Imperial College, London, and Dr. Arata Ideta, Principal of the Agricultural School, Yamaguchi-ken, Japan, who made extended trips through the country visiting colleges and experiment stations. There were many other foreign scientists interested in pathology who were brought into close touch with American pathologists through such visits. The Society was represented through its president, Dr. F. D. Fromme, at an international meeting, the first Pan-Pacific Food Conservation Conference, held in Hawaii during the first half of August.

Popular Articles on Plant Pathology. The Advisory Board has given consideration to the development of some agency for the preparation and publication of popular articles about plant diseases and the science of plant pathology. There are numerous popular articles appearing in the daily, weekly, and monthly press on subjects relating to chemistry, astronomy, geology, entomology, and other sciences, but very few on plant pathology. The advantage to our profession of having the public frequently, adequately, accurately, and clearly informed on this subject must be obvious to all plant pathologists.

After approval by the Council, the Board has appointed Dr. W. A. McCubbin to consider the possibility of developing such a service and, if conditions warrant, to initiate it. Dr. McCubbin is empowered to associate with him in this work such assistants as he may require.

Plant Disease Names. A committee on Plant Disease Names was appointed by the Society several years ago and it reported a year later, but while certain principles upon which such names may be based were approved, the names submitted by the committee were not adopted by the Society. No further action has been taken by that committee.

Inasmuch as the Plant Disease Survey of the U. S. Department of Agriculture, in the preparation and publication of reports on plant diseases, must apply common names to plant diseases and, in the application of such names, is guided by the principles adopted by the Society, and inasmuch as the Society is well represented in the Office of Plant Disease Survey through collaboration of members, the Advisory Board has recommended to the Council that this Office be recognized as the agency of the Society in the perfection of principles for naming plant diseases and in securing the more general adoption of names based on such principles.

Special Research and Investigational Projects. The Arthur rust project which was started September 1, 1922, has been actively carried on during the past eighteen months and at the present time seven out of the proposed eleven chapters have been written in preliminary form. Two of these chapters have been revised and practically put into final form. The remaining four unwritten chapters are expected to be completed by July 1. The book will shortly thereafter be ready for publication or as soon as the illustrations can be secured. The financial support of this project is satisfactory up to July 1, 1925, and it is thought that this will see the work nearly completed. Dr. F. D. Fromme spent the month of January on this work and Dr. C. R. Orton spent the month of June on it.

Very little has been done on the seed-borne parasites project beyond verifying references and getting the list of seed-borne parasites ready for publication. The American Seed Trade Association has been interested in having an investigation made of the problems of control of seed-borne diseases, and plans for such an investigation to be conducted through the Crop Protection Institute were prepared by the committee. The Association, however, decided to postpone making the necessary expenditure until a later date. Drs. C. R. Orton, M. T. Munn, and M. F. Barrus are members of this committee.

The Committee on Standardized Media for Fungous Cultures consists of Drs. G. H. Coons and L. M. Massey. Some work has been started by the committee and it wishes to report progress.

Investigations on Plant Diseases and on Fungicides Conducted Through the Crop Protection Institute. The cereal seed treatment project is cooperative between the American Phytopathological Society and the Crop Protection Institute. Drs. E. C. Stakman and C. R. Orton are members of the committee in charge. The principal object of the work this year was to determine the comparative effectiveness of certain dust and liquid fungicides in the control of covered smuts of wheat, oats, and barley. Investigations were carried on cooperatively with stations in Idaho, Washington, Ohio, Pennsylvania, New Jersey, Minnesota (4 stations), Quebec, Ontario, and Manitoba. The results this year indicate copper carbonate dust to be the most satisfactory fungicide for the control of bunt in wheat and formaldehyde for the control of oat smut. The results of the treatment for the control of barley smuts were inconclusive, owing to the small number of trials and to a low percentage of smut in the checks. A complete report of the work will be submitted to the Crop Protection Institute.

Scalecide investigations are being actively continued along the same lines as last year. Extensive measurements on trunk, twig, and leaf growth and on yield have shown no differences from check trees at the present time. There is no positive evidence yet as to the effect of Scalecide on fire blight control. Dr. C. R. Orton has served as chairman of the committee for the Crop Protection Institute.

The crown gall investigations project was definitely organized on May 10, 1924, with an advisory committee consisting of Drs. I. E. Melhus, as Chairman, G. W. Keitt, and M. F. Barrus. The men chosen as investigators are Dr. A. J. Riker and Mr. L. W. Boyle, of Madison, and Mr. J. H. Muncie, of Ames. Part of the work is being conducted in Wisconsin and part in Iowa. The project as laid out at present will run for two years for the maintenance of which the sum of $12,000 has been secured from the American Association of Nurserymen, individual nurserymen, the Iowa Agricultural Experiment Station, and the Wisconsin Agricultural Experiment Station.

Sulfur investigations are being concluded so far as the present project is concerned. The services of the two investigators expired during September, and reports of their work have been submitted to the project committee consisting of Drs. G. H. Coons, C. R. Orton, and P. J. Parrott.

The Furfural Investigations Committee consists of Drs. C. R. Orton, E. C. Stakman, and I. E. Melhus. Extensive tests of this material, which is one of the aldehydes, has been made at the Iowa Experiment Station. It has apparently fungicidal properties similar to formaldehyde in the treatment of Rhizoctonia on potatoes, but dissimilar to it in its effect on cereal seeds. The investigation at this stage has indicated that the material has fungicidal properties which will be studied further.

''Certain aspects of some copper salts'' is the name of the most recent project to be accepted by the Crop Protection Institute. The signatures of three companies have been secured to an agreement, namely, Nichols Copper Company, the Balbach Metals Corporation, and the Goldsmith Bros. Smelting and Refining Company. The sum assumed by them totals $3,000 a year for a period of two years. The cooperation of the Boyce Thompson Institute has been obtained in providing space and facilities for the copper studies, placing them under the supervision of Dr. Kunkel and Dr. Kraybill. Dr. William Crocker has accepted chairmanship of the committee and one other member, Dr. N. J. Giddings, has been appointed.

At the final meeting of the 1924 Advisory Board on January 1, 1925, Dr. M. F. Barrus was elected chairman for the ensuing year.

REPORTS OF OTHER COMMITTEES

Auditing Committee. The Auditing Committee, George L. Peltier and Charles Chupp, reported as follows:

We, the undersigned, have examined the books of the American Phytopathological Society (receipts and expenditures) and have not only found them correct, but prepared in the most excellent manner. We thank Dr. R. J. Haskell and Miss Mary G. Van Meter for their care in conducting this business.

We, the undersigned, have examined the books of PHYTOPATHOLOGY (receipts and expenditures) and have found them to be correct in every detail due to the excellent services of Dr. R. J. Haskell and Miss Mary G. Van Meter. We would suggest that the annual dues be paid in such a form that their face value will not be less than four dollars in American money.

Committee on Codifycation of Rules. The chairman of the committee, Dr. G. R. Lyman, submitted a set of standing rules of the Society together with proposed amendments to the constitution. On account of their length, the rules were adopted without being read and it was moved that they be published in PHYTOPATHOLOGY. These rules together with the proposed amendments to the constitution will appear in connection with the list of members of the Society in some one of the 1925 numbers of PHYTOPATHOLOGY.

Crown Gall Committee. I. E. Melhus presented the report for the crown gall committee as follows:

The crown gall work is under the immediate direction of a committee appointed by the Crop Protection Institute, consisting of: Dr. I. E. Melhus, Iowa State College, Ames, Iowa; Dr. Geo. Keitt, University of Wisconsin, Madison, Wisconsin, and Dr. M. F. Barrus, Cornell University, Ithaca, New York. The sources of funds are the National Nurserymen's Association, Iowa State College, and University of Wisconsin.

This committee met in Chicago last May with Professor W. C. O'Kane, Chairman of the Crop Protection Institute, and outlined the attack of the problem, budgeted its money allotment, and selected three research men, Mr. J. H. Muncie, stationed at Ames, Iowa, under the direction of Dr. I. E. Melhus; and Dr. A. J. Riker and Mr. L. W. Boyle located at Madison, Wisconsin, under the direction of Dr. Geo. Keitt.

The Crown Gall Committee is persuing its studies along four major lines as follows: (1) the effect of crown gall and hairy root on young trees, (2) the life history of the bacterium causing the disease, (3) the differential diagnosis of crown gall symptoms, and (4) the control of the disease in the nursery and orchard. It is recommended that the Crown Gall Committee be continued.

Committee on Plans for International Congress of Plant Sciences. The organization committee on the international congress of plant sciences has now practically completed the essential machinery of the permanent organization. This will consist of an executive committee composed of B. M. Duggar, H. C. Cowles, and H. H. Whetzel who, together with an executive secretary for each section, will constitute the general organization committee. Practically all of the societies interested in the international congress have designated one of their members to act as executive secretary. The duty of each executive secretary is to work up the program for papers to be presented at the scientific sessions which will consist of three half-day sessions. The congress is to be held at Cornell University, Ithaca, New York, the third week of August, 1926. I understand that the American Phytopathological Society has designated Dr. Ronald Reddick as their executive secretary and I am writing him in regard to his duties in this connection.—H. H. WHETZEL.

Joint Committee on Nomenclature. The Committee recommends the adoption and uniform use of the following terms in their Latin forms as here defined for species and subordinate groups of fungi, *viz.*, species, varietas, and forma.

Species. This term should be applied to a group of individuals which can be segregated on the basis of morphological characters of such a nature as to be applicable and determinable by mycologists and pathologists in general and will be available for general, practical taxonomic purposes.

Varietas. This term should be applied to a group of individuals of next lower rank than a species and should be designated by a trinomial. A varietas should also be distinguished by morphological characters, but such as are less constant and less important than those used for specific segregation.

Forma. This term should be applied to a subdivision of a species or variety which is characterized and distinguished primarily by physiological instead of morphological characters, although in some cases there may be very minor morphological characters distinguishable by intensive study. The segregation should be made, however, primarily on the basis of physiological behavior. Formae are to be designated by Arabic numerals, for example: *Glomerella lindemuthiana* F. 1; *Puccinia graminis tritici* F. 1.

It is recommended that hereafter the term forma be applied only to physiological groups, as indicated, and not to morphological groups or segregations.

It is recommended that the term "physiologic" or "physiological" or its abbreviation "P" be prefixed to avoid any confusion with morphological forms which will probably continue to be recognized to some extent. The complete and approved designation then would be, for example, *Puccinia graminis tritici P. F. 1.*

The above report signed by C. R. Ball for the American Society of Agronomy, H. S. Jackson for the Mycological Section of the Botanical Society of America, E. C. Stakman for the American Phytopathological Society, and C. F. Shear for all three societies was adopted and the committee discharged.

Pure Culture Supply Project. The chairman of the Committee of Pure Culture supplies, Dr. C. L. Shear, reported that, through the Division of Agriculture and Biology and the Division of Medical Sciences of the National Research Council, a committee had been organized including representatives of the American Bacteriological Society and the American Phytopathological Society. Through the efforts of this Committee the sum of $24,000.00 has been contributed by the Rockefeller Foundation for the support of this project for the next five years. At the request of the National Research Council, the carrying out of this project will be placed in the hands of a Committee representing the Society of American Bacteriologists, the Society of Medical Pathologists and Bacteriologists, the Zoological Society, the American Phytopathological Society, and the McCormick Memorial Institute of Chicago which is to take charge of the distribution of the cultures. These are expected to include eventually all microbiological organisms which can be supplied in pure culture. A catalog of the cultures which are available will be prepared as soon as practicable, giving prices and other information concerning the work. The most active cooperation of all biologists interested in this project is solicited.

This report was adopted and a resolution of appreciation and thanks to the Research Council approved unanimously. Dr. C. L. Shear was then elected the representative of this Society on the proposed committee to take charge of this project.

Committee on International Phytopathological Publications and Relations. Doctor Shear, Chairman of this Committee, reported that, in accordance with instructions and authority given the Committee by the Society at the Cincinnati meeting, arrangements have been made with Dr. H. M. Quanjer of the Phytopathological Institute, Wageningen, Holland, whereby he will act as the Editor of PHYTOPATHOLOGY for Europe, solicit subscriptions, and receive and transmit papers offered for publication in the Journal. Such papers are to be accepted in English, French, or German language, the total number of pages of such papers from Central European authors not to exceed 100 during the year. Special subscription rates have been offered in those European countries whose currency is below par. The rate according to this plan for the calendar year 1925 has been adjusted on the basis of European exchange. According to this the prices in the different countries will be as follows:

Albania	$2.50	Latvia	$2.50
Austria	2.50	Lithuania	2.50
Belgium	2.50	Netherlands	5.35
Bulgaria	2.50	Norway	2.92
Czechoslovakia	2.50	Poland	5.47
Denmark	3.61	Portugal	2.50
Esthonia	2.50	Rumania	2.50
Finland	2.50	Russia	2.50
France	2.50	Serbs, Croats, Slovenes	2.50
Germany	2.50	Spain	3.82
Great Britain	5.00	Sweden	5.46
Greece	2.50	Switzerland	5.47
Holland (Netherlands)	5.35	Turkey	2.50
Hungary	2.50	Ukraine	2.50
Italy	2.50	Yugoslavia	2.50

The Committee suggested a substitute title for the Journal beginning January 1, 1925, as follows:

"Phytopathology, an International Journal
and Official Organ of the American
Phytopathological Society"

This report was received and adopted and the Committee discharged.

Resolutions Committee. The American Phytopathological Society wishes to express its appreciation to the local committee who made arrangements for the convenience of the Society during this meeting. They especially appreciate the courtesy of Dr. William A. Taylor and the members of the staff of the Bureau of Plant Industry in offering facilities for visiting and inspecting the offices and laboratories, as well as the experimental station at Arlington.

The reports of the editor and the secretary-treasurer showing the excellent condition in which the Society now finds itself are especially gratifying and the Society takes this opportunity to express its sincere appreciation of the unselfish and efficient services of its officers.

The above resolutions were adopted. (See also resolutions at end of this report.)

ACTION OF THE COUNCIL

In addition to the appointments already mentioned in this report under the heading "Officers and Representatives," the Council reported the following actions which were approved by the Society.

It is recommended that there be established a standing committee on necrology to consist of three members whose terms of office shall be three years, such committee to be appointed by the Council.

It is recommended that the program committee arrange for a half-day session at the Kansas City meeting next year on the subject of the teaching of plant pathology.

Donald Reddick was appointed to serve as Executive Secretary for the Phytopathological Section of the International Botanical Congress of 1926.

E. C. Stakman and F. J. Schneiderhan were appointed to take charge of the preparation of a report of the Washington meeting.

MISCELLANEOUS BUSINESS

The secretary's report of last annual meeting as printed in the April, 1924, number of PHYTOPATHOLOGY was adopted.

It was voted to hold the next annual meeting of the Society at Kansas City in conjunction with the American Association.

In view of the fact that but $21.00 out of the original $105.00 remains for sending gratis copies of PHYTOPATHOLOGY to European pathologists and institutions, and because of the reduced rates that are now being offered them, the Society voted to cut down the present list and spend only $21.00 for these free copies during 1925.

Announcement was made of the first award of the Eunice Rockwood Oberly Memorial Prize. The prize was awarded to Mr. Max Meisel, formerly of the Science Division of the New York Public Library, for the first volume of his extensive bibliography on American natural history, published in the fall of 1924 by the Premier Publishing Company, 626 Broadway, Brooklyn, New York.

The Council was given power to act for the Society concerning any contribution that should be made to assist in financing BOTANICAL ABSTRACTS during 1925.

R. J. HASKELL, *Secretary*

PHYTOPATHOLOGY

An International Journal
Official Organ of the American Phytopathological Society

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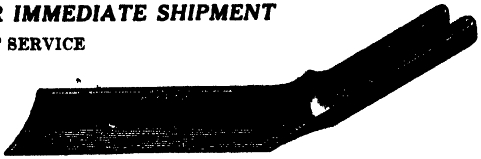

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PHYTOPATHOLOGY

VOLUME XV NUMBER 6

JUNE, 1925

ARSENICAL INJURY OF THE PEACH[1]

C. M. HAENSELER AND WM. H. MARTIN

WITH FOUR FIGURES IN THE TEXT

During the past several years there has occurred, in various sections of New Jersey, an unusual amount of leaf and twig injury to the peach, due apparently to arsenate of lead used in the spray mixtures. Although leaf injury and defoliation from the use of toxic sprays is not uncommon, severe injury to the twigs was not noted in this state until recent years. In 1922 twig injury was severe in South Jersey, where it occurred in the form of cankers on the new and the one year old wood.

In 1923 a mild form of this same type of injury was observed in a number of sprayed orchards, but no cases of serious damage were reported. During 1924, however, complaints of severe defoliation and wood cankering came in from all parts of the state. In Burlington, Camden, Gloucester, Middlesex and Monmouth counties, New Jersey, a number of orchards were examined where the injury was very severe. In one orchard, for example, which had been sprayed with 1½ lbs. arsenate of lead in 50 gals. water, the fruit and leaves had prematurely fallen and the new and one year old woods were severely cankered. In another orchard, sprayed with atomic sulfur and arsenate of lead, the leaves showed large burned areas and most of the leaves had fallen. The new and the one year old twigs were also severely cankered and on some trees branches were broken off at the cankered crotches. In still another orchard where dry-mix (8 lbs. sulfur, 4 lbs. lime and 1½ lbs. arsenate of lead to 50 gals. water) was used, there was only a very slight leaf injury and very little defoliation but the new and one year old twigs were severely cankered. These observations and those made in other orchards showed that the injury occurred in several forms and that the relative amount of each form varied in the different orchards. In some the injury was most prevalent on the leaves; in others, the leaves showed little injury while the twigs were severely cankered.

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[1] Paper No. 206 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Plant Pathology.

Furthermore, the appearance and location of the twig cankers varied considerably with the age and condition of the twigs and with the severity of the injury. In view of the unusual prevalence and severity of these cankers in New Jersey, a series of spray tests have been conducted the past three years to determine the relation of various spray mixtures to the injury.

Before entering into a discussion of the experimental spray tests it may be well to give a detailed description of the various types of injury resulting from arsenical burning on the peach The injury occurs mainly on the leaves, young shoots and the one year old wood, and occasionally on the fruit On the leaves it appears as brown, circular, necrotic areas usually ⅛ to ¼ inch in diameter (Fig. 1), or a narrow strip of dead tissue along the leaf margin. These dead areas frequently break away from the living portion of the leaf, causing a ragged appearance, or "shot hole" somewhat similar to that caused by the bacterial leaf-spot organism Severely affected leaves usually fall within a week after the injury becomes

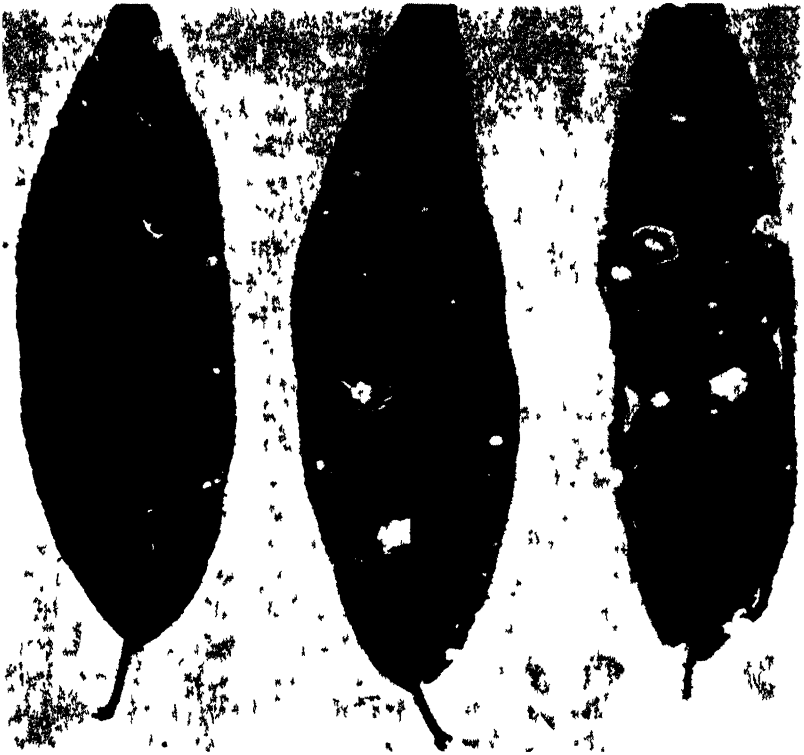

FIG. 1. Leaves showing typical symptoms of spray injury.

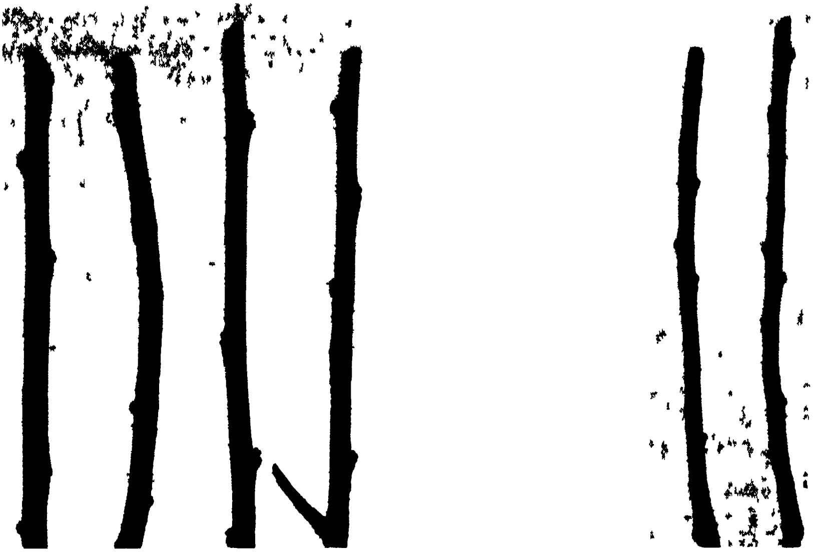

FIG. 2. Young twigs showing bud cankers due to spray injury.

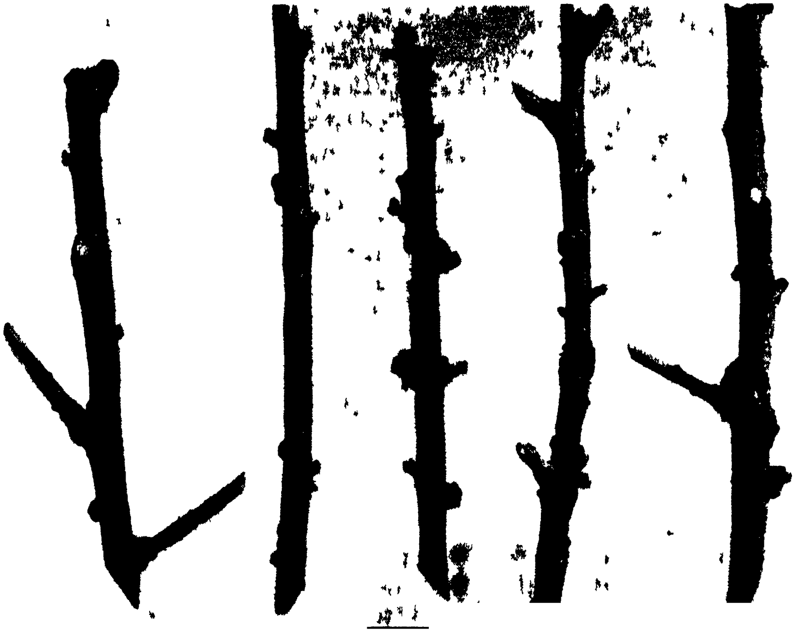

FIG. 3. One year old twigs showing cankering and gummosis due to spray injury.

visible, although a few may remain on the tree all summer. There may also be considerable premature defoliation of green or yellow leaves which show no apparent injury. In such cases the leaf drop is not infrequently associated with a cankering of the nodes of the new shoots. The older leaves are more subject to injury than the younger ones, the latter frequently remaining unaffected.

On the new growth the injury appears mainly at the two or three oldest nodes (Fig. 2) and at the juncture between the new and old wood, rarely along the older internodes. The nodal or bud cankers appear as very conspicuous, well-defined, brown, dead areas 1/8 to 3/8 inch long and generally about half encircling the twig. They always occur on the bud side of the node, either immediately below or completely surrounding the bud, which is usually killed. The tissues immediately surrounding these cankers are usually healthy but may show a conspicuous reddening of the bark. The cankers at the base of the new wood are brown, necrotic bands, usually 1/8 to 1/4 inch long, completely surrounding the twigs. Such girdling cankers often weaken the shoots, causing the newly developed leaves to become pale and rolled inward. In severe cases some of the shoots may die or break off at the girdled region, giving the tree a very ragged appearance. Cankers may also occur on the internodes, but these are rare and seem to be associated with slight abrasions. These internodal cankers are variable in size and shape and seldom have a definite outline.

On one year old twigs, with brown, corky bark, the first sign of injury appears as slightly darkened blotches, mainly along the upper or more exposed surface. If the first layer of the bark is removed at this stage, the cankered areas show a reddening of the tissue immediately beneath the corky bark and a water-soaked condition of the deeper layers. The later development of the canker depends on the tissues affected. If the burning affects only the cortex, the twigs continue to enlarge; and the injured cortex cracks and partly sloughs off, giving a very shaggy appearance. If the cambium is killed, the cankered areas become sunken, due to the growth of adjacent tissues. These areas finally crack, and in wet weather exude large amounts of gum (Fig. 3). In case of severe burning, many twigs have spaces 6 in. to 1 ft. long where the cambium has been destroyed on the more exposed side. Occasionally a canker completely girdles a twig, causing a slow dying; but, in most cases, sufficient uninjured cambium remains to keep the twig alive and bring about a slow recovery. The bark, however, remains very rough for some time, and old cases of injury can often be recognized by these shaggy-barked limbs (Fig. 4, center). This healing process usually brings about a complete recovery along the main part of the limbs, but where the cankers occur in crotches the injury is often permanent. Cankers at such points seem to be unable to heal properly and, as a result, the injured crotches remain very weak and often break from wind strains or from heavy crops (Fig. 4, left).

On small green-barked twigs, one year old, the injury does not appear as cracking or gumming cankers as in the case of larger corky-barked twigs. Here it takes the form of dark blotches, usually from ¼ to ½ inch long and half encircling the twigs. They are generally located at the nodes, but in severe cases the greater part of the twig may be affected. The nodal cankers have a rather definite outline and stand out very conspicuously against the green background. On small, weak, one year old twigs, such as appear so abundantly towards the center of poorly pruned trees, this type of injury becomes so severe that the twigs are killed outright, giving the general appearance of a severe case of brown-rot twig blight.

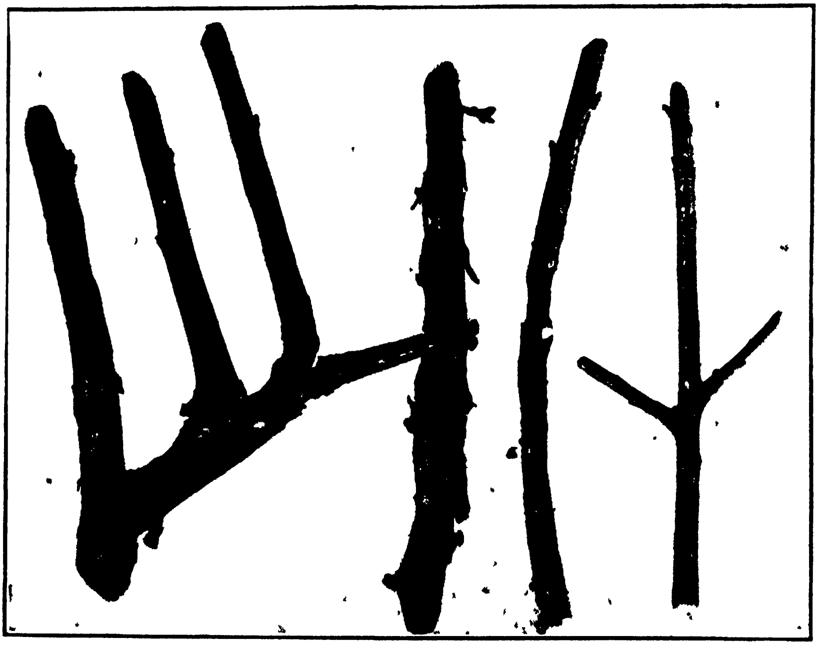

FIG. 4. Left. Splitting of one year old crotches following spray injury. Center. One year old twigs showing roughened bark following spray injury. Right. One year old twig from unsprayed check tree showing smooth bark.

The young fruit seems to be more resistant to spray injury than the leaves and twigs. Orchards have been observed which were from 50 to 75 per cent defoliated, and where all of the one year old twigs were severely cankered as a result of arsenical injury, and yet the fruit which remained on the trees showed no sign of burning. The fruit is always reduced in size, however; and, where defoliation is severe, the quality is greatly reduced.

SPRAY TESTS IN 1922

The spray tests in 1922 were made in cooperation with the Horticultural Department of the New Jersey Agricultural Experiment Station.

Since atomic sulfur had been used on most of the injured orchards observed in 1922, this material was tested in various combinations with lime and arsenate of lead in order to determine, if possible, the exact cause of the burning. A single tree was used for each mixture and only one spray application was given on July 26, when the new wood had completed its most rapid growth. The spray mixtures used and the results obtained are given in table 1.

The first injury was observed on August 10, 15 days after spraying. By August 18 the burning had apparently reached its maximum, and on this date a careful examination was made of all the trees. It will be observed from table 1 that the unsprayed tree (No. 12) and all trees receiving atomic sulfur, self-boiled lime sulfur, or dry-mix, but no arsenate of lead (Nos. 1, 8, 10 and 11), showed no injury of any kind. On the other hand, the pure arsenate of lead spray (No. 5) and the arsenate of lead-lime sprays (Nos. 6 and 7) caused severe burning. It is of interest to note that where lime and arsenate of lead were used (Nos. 6 and 7) the injury was somewhat more severe than where the same amount of arsenate of lead was used alone (No. 5). It will also be noted that 1½ lbs. arsenate of lead, with 5 lbs. atomic sulfur in 50 gals. of water, caused considerable injury (No. 2), but when 5 lbs. lime was added to this combination (No. 3) the burning was almost entirely prevented. Where the arsenate of lead was increased to 2½ lbs. to 50 gals. (No. 4), however, the burning occurred regardless of the fact that 5 lbs. of lime was used. Arsenate of lead, 1½ lbs. to 50 gals. of standard self-boiled lime sulfur (No. 9), caused only a trace of injury.

In summarizing the results of this test, we see that atomic sulfur, self-boiled lime sulfur, and dry-mix, when used without arsenate of lead, caused no injury, while arsenate of lead used alone or with lime, or used in excess in sulfur-lime-lead mixtures, caused considerable leaf and twig injury from a single late application.

SPRAY TEST IN 1923

In 1923 a test was made in which sulfur, lime and arsenate of lead were used alone and in the various combinations indicated in table 2. One application was made on August 7 on vigorous, four year old trees. The

TABLE 1.—*Results of Spray Tests in 1922*

TREATMENT[a] Lbs. materials in 50 gals. water		OBSERVATIONS
1. Atomic Sulfur	5 lbs.	No injury.
2. Atomic Sulfur Arsenate of Lead	5 lbs. 1½ "	Severe cankering and gummosis both on 1 yr. wood and older portions of new wood. No spotting of leaves; heavy drop.
3. Atomic Sulfur Stone Lime Arsenate of Lead	5 lbs. 5 " 1½ "	Slight cankering, restricted to buds. Slight leaf drop.
4. Atomic Sulfur Stone Lime Arsenate of Lead	5 lbs. 2½ " 1½ "	Considerable cankering. Heavy leaf drop.
5. Arsenate of Lead	1½ lbs.	Severe cankering of old and new wood. Leaves showing large brown areas and shot holes. Heavy drop.
6. Arsenate of Lead Stone Lime	1½ lbs. 5 "	Cankering more severe than on 5. Shot holes abundant. Leaf drop on some twigs amounting to 90 per cent.
7. Arsenate of Lead Stone Lime	1½ lbs. 2½ "	Injury similar to 6 but less severe.
8. Sulfur Stone Lime (Self-boiled)	8 lbs. 8 "	No injury.
9. Sulfur Stone Lime Arsenate of Lead (Self boiled)	8 lbs. 8 " 1½ "	A very small number of cankers observed on new wood.
10. Sulfur Hydrated Lime Kayso (Dry mix)	8 lbs. 4 " ½ lb.	No injury.
11. Sulfur Hydrated Lime Kayso (Dry-mix)	2 lbs. 4 " ½ lb.	No injury.
12. Check—unsprayed		No injury.

[a] One spray application made July 26, 1922.

season was hot and dry, and at the time of spraying the trees had finished their most active spring growth. On August 14, seven days after spraying, no traces of injury were observed. On the 17th, ten days after spraying, a few gumming cankers were noted on the blocks where arsenate of lead alone (No. 1), arsenate of lead and sulfur (No. 4), and where arsenate of lead, sulfur and a small amount of lime (No. 8) were used. The injury did not develop further or appear on any other blocks later in the season. The injuries secured in this test were so slight that a comparison of the relative toxicity of the various mixtures would be of little value, but it is significant to note that here, as in the 1922 test, injury occurred only where arsenate of lead was used. It is also of interest to note that arsenate of lead alone, used at the rate of 1½ lbs. to 50 gal. of water, caused only slight wood cankering and defoliation, while in 1922 this same spray gave severe injury (table 1, plot 5).

These results coincide with the field observation noted above, which showed that spray injury was much more general in 1922 than in 1923. This difference in the amount of injury obtained in these two years from the same type of spray is doubtless closely associated with differences in seasonal conditions, the wet season of 1922 apparently being more favorable for arsenical injury than the dry season of 1923. It should also be noted in this connection that the results of tests conducted in 1924 indicate that the maturity of the wood and the vigor of the tree are important factors in determining the susceptibility of a tree to arsenical injury. The dry season in 1923, the relatively mature condition of the wood at the time of spraying, and the vigorous condition of the trees all, no doubt, were important factors in accounting for the small amount of injury secured in this test.

TABLE 2.—*Results of Spray Test in 1923*

TREATMENT[a] Lbs. material in 50 gal. water.		OBSERVATIONS—AUG. 17
1. Arsenate of Lead	1½ lbs.	Slight cankering and gummosis on one year old wood.
2. Sulfur	8 lbs.	No injury.
3. Hydrated Lime	4 "	No injury.
4. Arsenate of Lead Sulfur	1½ " 8 "	Slight cankering and gummosis on one year old wood.
5. Arsenate of Lead Hydrated Lime	1½ " 4 "	No injury.
6. Arsenate of Lead Sulfur Hydrated Lime	1½ " 8 " 4 "	No injury.
7. Arsenate of Lead Sulfur Hydrated Lime	1½ " 8 " 2 "	No injury.
8. Arsenate of Lead Sulfur Hydrated Lime	1½ " 8 " 1 lb.	Slight cankering and gummosis on one year old wood.

[a] One application made on Aug. 7.

SPRAY TESTS IN 1924

In 1924 a more extensive test was conducted on three-year old trees, which had not previously been sprayed, in order to compare dry-mix sprays containing various amounts of sulfur, lime and arsenate of lead, and to determine the time of year when the trees are most subject to injury. The test orchard was divided into blocks of four trees each, three of which were sprayed and the fourth left as a check. Three spray applications were made, on June 7, June 23 and July 11, the first being applied a few days after the shucks had fallen. The various treatments and the results of observations made on July 22, at which date the injury had reached its maximum, are given in table 3.

It will be observed from the notes given in table 3 that 1½ lbs. arsenate of lead to 50 gals. of water (Plot 10) caused more severe injury than any other spray used. Where 4 lbs. of hydrated lime was added to the 1½ lbs. arsenate of lead (Plot 1) the burning was considerably reduced but was still severe. Where arsenate of lead and sulfur were used (Plot 5), the burning was practically the same as with arsenate of lead alone. Varying the amount of sulfur in the mixtures containing sulfur, lime and arsenate of lead had little influence on the toxicity of the spray (Plots 2, 3 and 4), but where the amount of lime was varied (Plots 6, 7 and 8) the degree of injury decreased as the amount of lime was increased. On plot 6, for example, where 2 lbs. of hydrated lime was used to 8 lbs. of sulfur and 1½ lbs. of arsenate of lead, the twig cankering and gummosis was very conspicuous; while on plot 8, where the lime was increased to 6 lbs., there was no gummosis and only a trace of cankering.

It is also of interest to compare plots 7 and 9, where the dry-mix contained different amounts of arsenate of lead. On plot 7 where the spray mixture contained 8 lbs. sulfur, 4 lbs. lime and 1½ lbs. arsenate of lead, the proportion recommended for peaches in 1922 and 1923, a moderate amount of injury occurred but it was by no means conspicuous. On plot 9 where the arsenate of lead was increased to 2½ lbs. injury was very severe. The standard self-boiled lime sulfur with 1½ lbs. of arsenate of lead (Plot 11), which was included in the test as a comparison check, caused no trace of injury either to leaves or twigs.

It will be seen from the results in plots 12, 13 and 14, where the first, second, and third spray applications respectively were omitted, that the time of application greatly influences the amount of injury caused by the toxic spray mixtures. In plot 12, where the first spray (June 7) was omitted, there was only a trace of burning, while in plots 13 and 14, where the first spray was applied but the second (Plot 13) or third (Plot 14) omitted, the injury was just as severe as in plot 9 which received three applications of the same spray mixture. This would indicate that the peach is much more subject to spray injury in the early spring when the trees are growing rapidly than in mid-summer when the cambium is less active.

In conclusion it should be stated that the injury obtained on the trees in the experimental plots, which was evidently due to lead arsenate used in the spray mixture, was similar in every respect to the injury which was so prevalent in the orchards in South Jersey in 1922 and in all parts

TABLE 3.—*Results of Spray Tests in 1924*

TREATMENT[a] Lbs. materials in 50 gals. water		OBSERVATIONS—JULY 22
1. Hydrated Lime Arsenate of Lead	4 lbs. 1½ "	Slight amount of cracking and gummosis on 1 yr. wood. 50 per cent of twigs cankered on new wood. From 1 to 2 bud cankers on each. Leaf burning slight.
2. Sulfur Hydrated Lime Arsenate of Lead	4 lbs. 4 " 1½ "	Trace of burning and gummosis on 1 yr. wood. On weak trees 30 per cent of twigs showing bud cankers on new wood. On vigorous trees bud cankers rare. No conspicuous leaf burning.
3. Sulfur Hydrated Lime Arsenate of Lead	6 lbs. 4 " 1½ "	Trace of burning and gummosis on 1 yr. wood. A few bud cankers. No conspicuous leaf burning.
4. Sulfur Hydrated Lime Arsenate of Lead	8 lbs. 4 " 1½ "	Injured to same extent as Nos. 2 and 3.
5. Sulfur Arsenate of Lead	8 lbs. 1½ "	On 1 yr. wood, every twig cankered and abundant gummosis. On vigorous tree, 20 per cent of twigs with bud cankers on new wood. On weak trees 50 per cent. No leaf burning.
6. Sulfur Hydrated Lime Arsenate of Lead	8 lbs. 2 " 1½ "	On 1 yr. wood every twig severely cankered. Much less gummosis on thrifty than on weak trees. On vigorous trees 10 per cent of twigs with bud cankers on new wood. On weak trees 70 per cent. No leaf burning.
7. Sulfur Hydrated Lime Arsenate of Lead	8 lbs. 4 " 1½ "	On 1 yr. wood approximately 25 per cent twigs cankered with small amount of gummosis. Bud cankers on new wood rare.
8. Sulfur Hydrated Lime Arsenate of Lead	8 lbs. 6 " 1½ "	On 1 yr. wood, cankers rare with no gummosis on thrifty tree, on weak trees a few more cankers but no gummosis. Trace of bud cankers on new wood on weak trees, none on vigorous.
9. Sulfur Hydrated Lime Arsenate of Lead	8 lbs. 4 " 2½ "	On 1 yr. wood on thrifty tree every twig burned with slight gummosis. Weak trees severely cankered with heavy gummosis. Few bud cankers on new wood on a vigorous tree, on weak tree 75 per cent of twigs showed bud cankers.
10. Arsenate of Lead	1½ lbs.	On 1 yr. old wood every twig severely cankered with abundant gummosis. Most twigs cankered for entire length. 50 per cent of twigs with bud cankers on new wood. Injury more prevalent than on any other plot.
11. Sulfur Lime Arsenate of Lead (Self-boiled)	8 lbs. 8 " 1½ "	No injury of any kind.
12. Sulfur Hydrated Lime Arsenate of Lead 1st. spray omitted	8 lbs. 4 " 2½ "	On 1 year wood on one tree 3 small cankers, none on other trees. No bud cankers.
13. Same as No. 12. 2nd. spray omitted		On 1 year wood all twigs severely cankered with heavy gummosis. Some bud cankers.
14. Same as No. 12. 3rd. spray omitted		Cankered to same extent as No. 12.

[a] Applications: June 7, 23, July 11. Self-boiled lime-sulfur used on plot 11. All other spray materials prepared by the dry-mix method, using ½ lb. calcium caseinate per 50 gals. spray mixture.

of the state in 1924. Reports have reached us that similar injury has occurred in orchards where no sprays or dusts were applied, but not a single authentic case of this kind has been observed. It is recognized that brown rot may cause an occasional gumming twig canker and that the bacterial leaf spot organism may cause serious defoliation; but a microscopic and cultural study of many twig cankers, and the results of spray tests conducted over a period of three years, lead us to believe that much of the premature defoliation and most of the twig cankering and gummosis so prevalent in New Jersey during the past three years have been due to the use of toxic spray materials and not to the brown-rot or leaf-spot organisms.

In the Hammonton section the bacterial leaf spot sometimes causes almost complete defoliation; but no cases have been observed, even in such severe infections, where a conspicuous twig cankering is associated with this bacterial disease.

The results of the three-year tests demonstrate conclusively that where arsenate of lead is used alone at the rate of 1½ lbs. to 50 gallons of water, or when used in excess with sulfur or lime or in combination of the two, severe injury may be expected both on the leaves and the twigs of the peach, especially from the early spray applications.

SUMMARY

1. Spray injury to the peach appear mainly as: 1, a leaf burning which frequently results in premature defoliation; 2, as necrotic areas at the older nodes of the new growth; 3, as cankers on the one-year old wood which cause a splitting of the bark and gummosis.
2. Atomic sulfur, flowers of sulfur, and lime alone caused no injury.
3. Powdered arsenate of lead, 1½ lbs. to 50 gals. of water, when used alone or in combination with atomic sulfur, sulfur, or lime, caused severe injury.
4. In mixtures containing sulfur, lime, and arsenate of lead, injury occurred only when the lime was appreciably reduced, or the arsenate of lead increased over the amounts generally recommended.
5. Self-boiled lime sulfur, 8–8–50, with 1½ lbs. of arsenate of lead caused no injury, while dry-mix, 8–4–½–50, with 1½ lbs. arsenate of lead caused injury.
6. In dry-mix, increasing the arsenate of lead to 2½ lbs. or reducing the lime to 2 lbs. made the mixture more toxic.
7. Weak trees were more subject to spray injury than vigorous ones.
8. Sprays applied early in the season caused more injury than those applied later.

NEW JERSEY AGRICULTURAL EXPERIMENT STATION,
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THE CITRUS STEM-END ROT "DIPLODIA"; ITS LIFE HISTORY AND RELATION TO SPHAEROPSIS MALORUM

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WITH PLATE VIII

The close relationship between the *"Diplodia"* which causes the destructive stem-end rot of citrus fruits and the *"Sphaeropsis"* which causes black-rot of apples has been pointed out at various times. Continued study of these two organisms has convinced the writers that their perithecial stages are even more similar than the pycnidial, but that they exhibit certain distinguishing cultural characters and that the pycnospores may be distinguished morphologically, even when grown on the same culture medium. The evidence on these points is presented in the present paper for the information of those who are engaged in the field study of the diseases caused by this important group of organisms and particularly in the hope that more material of the perfect stage will be collected, especially in the tropics, and critically studied.

In referring to the citrus end-rot organism, the name *Diplodia natalensis* Pole-Evans (2) will be used, as this seems to have become well established by the usage of students of citrus diseases in this country and Cuba (1, 3, and 7). For clearness in comparison, the apple black-rot fungus will be referred to by the name usually applied to its pycnidial stage *Sphaeropsis malorum* Peck.

EARLIER WORK

In discussing *Diplodia natalensis* on citrus in the Isle of Pines, Earle and Rogers (1) sum up the resemblance of this organism to *Sphaeropsis malorum* as follows: (Their statement is quoted in full, as the original publication does not appear to be generally accessible.)

> ". . . On the other hand Diplodia differs from Sphaeropsis only in having one septate or two-celled spores instead of a simple one-celled spore. At full maturity the spores of our fungus are two-celled with a heavy, dark brown, highly chitinized wall, which probably is accountable for their great resistance to the action of disinfectants. They are oval in outline, scarcely constricted and measure about 12–15 x 25–30 microns. On some media, however, they remain for a long time one-celled even after the wall is considerably chitinized. In this condition the fungus may easily have been described in this genus and must be studied before we can be sure that our fungus is not among them. In fact its resemblance to *Sphaeropsis malorum* Pk., the common black rot of apples, is so striking both from its cultural characters and from the fact that it causes a destructive rot of the fruit, and also destructive lesions on the bark, that the possibility of some connection between the two suggested itself. We soon found that our

Diplodia would rot down apples with all the symptoms of the Sphaeropsis rot. The pycnidia were even single and scattered—not clustered, as on citrus, and the spores remained long one-celled. Through the kindness of Dr. D. C. Babcock, of the Ohio station, a pure culture of *Sphaeropsis malorum* was secured. When inoculated into grapefruit it promptly produced a rot that was indistinguishable from the ordinary Diplodia rot. So far no pycnidia have developed, but this always requires a long time on grapefruit. In a long series of parallel cultures, on different media, the only observable difference in behavior was in the decided tendency to form prominent sclerotium-like masses on the part of the Diplodia. Somewhat similar mycelial masses were observed in some of the Sphaeropsis cultures, but they were much smaller and less conspicuous. Both strains blacken the substratum conspicuously about the fourth day after inoculation and neither seems inclined to form pycnidia in tube cultures. But for this difference in the tendency to form sclerotium-like bodies we should certainly be compelled to consider them identical. As it is we can only wait for further developments and present such evidence as we have on hand. So far as we know the ascus bearing stage of *Sphaeropsis malorum* has not been discovered. Neither have we, as yet, been able to connect the Diplodia with any ascus bearing form. The further discussion of this question, as well as that of an effective disinfectant for Diplodia spores, is reserved for some future publication.''

EFFECT ON THE FRUIT

It would be confusing if the resemblances between the two organisms here considered were confined to morphological characters. It is apparent, however, from the work of Earl and Rogers (1) that they have a somewhat similar effect both on apples and grapefruit when introduced by inoculation. Moreover, in the paper in which he describes *Diplodia natalensis,* Pole-Evans (2) calls attention to the fact that the fungus fruits more readily on apples than on citrus fruit. Indeed, the spores described in that paper are apparently those produced on inoculated apples.

As already pointed out by the present writers, the rather closely related *Botryosphaeria ribis chromogena* (4) is able to produce a rot of citrus fruits much resembling that caused by the citrus Diplodia itself. *B. ribis* is already known from South Africa and New South Wales and as both *B. ribis* and *S. malorum* are found generally distributed in the citrus region of Florida, it is well within the bounds of possibility that continued study will reveal the occasional presence of both *B. ribis* and *S. malorum* in decayed citrus fruits.

While it is of interest to note that these closely related fungi may cause a decay of citrus fruit closely resembling that caused by *Diplodia natalensis,* it will hardly be maintained that this fact in itself should be taken as indicating any relationship of the two fungi, especially since those most familiar with the diseases of citrus fruits are unable to distinguish the rot caused by *D. natalensis* from that caused by *Phomopsis citri* (7). Moreover, there can be no reasonable question that the fungus that causes the Diplodia end-rot is, in an overwhelming number of cases, not *Sphaeropsis malorum* but *Diplodia natalensis.* During the past three years, H. R. Fulton and J. R. Winston have furnished the writers, for study, numerous cultures of Diplodia isolated from decayed citrus fruit. In all cases these have proven to be *Diplodia natalensis* as the name is used in this paper.

Moreover, in a series of experiments recently made by Mr. Winston at Orlando, Florida, in which different lots of grapefruit were inoculated with pure cultures of these two fungi, *Diplodia natalensis* was decidedly more active as a cause of decay than *Sphaeropsis malorum.* Under the conditions of storage there used practically every fruit inoculated with *Diplodia natalensis* developed the typical "end rot" from which *Diplodia natalensis* was again isolated. *Sphaeropsis malorum,* on the other hand, did not grow at all in the grapefruit or grew only very slowly.

BEHAVIOR OF DIPLODIA NATALENSIS IN CULTURE

Pycnospore production. Earle and Rogers (1) call attention to the fact that neither *Sphaeropsis malorum* nor *Diplodia natalensis* formed pycnidia in culture in the Isle of Pines. Mr. J. R. Winston has informed the writers that pycnospores have been formed but rarely in a long series of cultures of *D. natalensis* in Florida and in the laboratory at Washington. Numerous investigators have had the same experience with *Sphaeropsis malorum.* Both fungi will, however, produce pycnospores abundantly in pure culture if given suitable environmental conditions. The writers have had excellent results in securing pycnospore production in pure cultures of both species in a well ventilated greenhouse at Arlington, Virginia, during the fall and winter months and in unheated wooden buildings at Wareham and Woods Hole, Massachusetts, during the summer (4, p. 590). Summer temperatures in Washington apparently are too high to permit the fungi to fruit readily in culture. They are apparently very similar in their requirements for pycnospore production but must differ somewhat, as for reasons not yet understood *D. natalensis* will sometimes fruit when cultures of *S. malorum* kept under the same conditions remain sterile.

Cultural characters. On many culture media and, especially while young, cultures of *Diplodia natalensis* and *Sphaeropsis malorum* are certainly very similar in appearance. As the fruiting period approaches, however, there is a marked tendency for the Diplodia to form what Earl and Rogers (1) term "sclerotium-like masses" on the surface of the medium. In the surface layers of these mycelial masses the pycnidia are formed. The pycnidia of *Sphaeropsis malorum,* on the other hand, are only very slightly raised above the surface of the closely matted mycelium. The appearance of fruiting cultures of these two fungi on corn meal in flasks is shown in plate VIII (Figs. D and E).

That the size of the sporocarp of *D. natalensis* varies with the host and the thickness of the bark on which it is grown has been pointed out in an earlier publication (6).

TEMPERATURE RELATIONS

A far more striking difference between cultures of *D. natalensis* and *Sphaeropsis malorum* than their superficial appearance is the wide difference in the temperature relations of the two fungi. Without attempting the all but impossible task of fixing definite optimum and maximum temperatures for the growth of these fungi, it may be noted that *Diplodia natalensis* will grow somewhat at 37° C., whereas *Sphaeropsis malorum* grows only very slowly at 31° C. Moreover, when grown on potato dextrose agar at temperatures above 32° C., many stains of *Diplodia natalensis* turn the medium a bright pink or red. The potato dextrose agar used was made as follows:

Clean pared potatoes were ground and weighed. Distilled water was then added in the ratio of 2 cc. of water to 1 gram of the potato. The mixture was kept in the ice box two hours, then put through gauze and a meat press. The juice was filtered through paper to remove the starch, steamed one hour, then filtered again through the cotton. After making up to the original amount, one and one-fourth per cent of shredded agar was added, and the mixture steamed for one and one-half hours. Two per cent dextrose was added before filtering through the cotton. The agar was then tnbed and autoclaved thirty minutes at fifteen pounds pressure.

PYCNOSPORES

In an earlier paper the writers (5) have shown that the perfect stage of *Sphaeropsis malorum* (*Physalospora malorum*) has been found on twenty-two host species in the eastern United States. It has also been twice found on citrus. The perfect stage of *Diplodia natalensis* has also been found at least once by the writers on citrus. The pycnospores of *Diplodia natalensis*, described in this section and in tables 1 to 3, were all grown in pure culture on corn meal in 100 cc. Erlenmeyer flasks from single ascospores; but they agree in all respects with pycnospores produced in culture from mycelium isolated from decayed citrus fruits.

Under the conditions of our work, pycnospores of *Diplodia natalensis* may be distinguished from those of *Sphaeropsis malorum* by differences in size and shape, in degree of septation, in color of the mature spores, and by the frequency with which hyaline spores are found. Tables 1 and 2 give the size and table 3 the shape as expressed by the ratio of length to width of what we regard as typical pycnospores of these fungi. Pycnospore measurements which are summarized in the first line of each of these tables

TABLES 1 TO 3.—*Comparative measurements of pycnospores of Sphaeropsis malorum and Diplodia natalensis from single ascopores in culture*

Table 1. Lengths in microns	Total number of spores	Number of spores having a given length															
		17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Sphaeropsis malorum from 22 different hosts	1169	10	11	10	99	45	164	152	164	230	116	96	31	22	14	4	1
Sphaeropsis malorum from citrus twigs	100				1	1	4	4	15	16	23	16	9	7	4		
Diplodia natalensis from citrus twigs	200				1	6	15	19	29	33	20	27	18	13	16	2	1

Table 2. Widths in microns	Total number of spores	Number of spores having a given width										
		7	8	9	10	11	12	13	14	15	16	17
Sphaeropsis malorum from 22 different hosts	1169	14	91	157	397	220	143	84	28	35		
Sphaeropsis malorum from citrus twigs	100			11	43	32	13		1			
Diplodia natalensis from citrus twigs	200					8	33	64	46	35	13	1

Table 3. Ratio of length to width	Total number of spores	Number of spores having a given ratio of length to width					
		1.5	2	2.5	3	3.5	4
Sphaeropsis malorum from 22 different hosts	1169	71	450	506	119	20	3
Sphaeropsis malorum from citrus twigs	100		20	65	15		
Diplodia natalensis from citrus twigs	200	39	158	3			

were made from pycnospores produced in pure culture from single ascospores of *Physalospora malorum* from the hosts discussed in earlier papers (4, 5), namely: *Acer* sp. five specimens; *Alnus* sp., two specimens; *Amygdalus* sp.; *Cercis* sp.; *Crataegus* sp.; *Diospyros* sp.; *Hicoria* sp., four specimens; *Liquidambar* sp., three specimens; *Liriodendron* sp.; *Lucuma* sp.; *Magnolia* sp.; *Melia* sp., three specimens; *Platanus* sp.; *Prunus* sp.; *Pyrus malus; Quercus* sp., six specimens; *Ribis* sp.; *Rubus* sp.; *Salix* sp., three specimens; *Sassafras* sp.; *Citrus* sp.; *Viburnum* sp., and *Vitis* sp. These were collected at various places in the eastern United States. It will be noted that while they differ but little in length, the extremes differing very little in the two species, there is a slightly larger proportion of long spores in *Diplodia natalensis.* The differences in width are more marked. Pycnospores of *Sphaeropsis malorum* measure from 7 to 15 microns in width, mostly 10 or 12, while those of *Diplodia natalensis* measure 11 to 17, mostly 13 or 14. The difference in width is of course reflected in the shape (Table 3), *Diplodia natalensis* showing a decidedly larger proportion of spores whose width is half or more than half of their length. Pycnospores of the two fungi are, however, more readily distinguished by color and septation than by size. Although septate pycnospores are occasionally found in *Sphaeropsis malorum,* they are, under the conditions of our work, exceedingly rare. One often examines a dozen slides well covered with mature pycnospores without finding a single septate spore. On the other hand, while hyaline spores of *Diplodia natalensis* are almost always one-celled, in the great majority of cases they develop a septum by the time the outer wall becomes colored. Mature (colored) one-celled pycnospores of *Diplodia natalensis* are not so rare as septate ones of *Sphaeropsis malorum,* but they are in a decided minority.

In general, spores of *Diplodia natalensis* remain hyaline until about the time they are discharged from the pycnidium. As a result, colorless spores of this fungus are abundant and may be found in almost any preparation. On the other hand, hyaline spores of *Sphaeropsis malorum* are comparatively rare. The pycnospores of this fungus apparently become colored very soon after they are detached from the sporophore, or, in some cases, even before they become detached. As pointed out by many writers, the cell wall of spores of *D. natalensis* appears to be very thick, even while the spores are hyaline. These various points are well shown by figures A and B of plate VIII.

A difference in the pycnospores of these fungi which is very readily detected but not easily described is the difference in the color and markings of the spore wall. Mature spores of *Sphaeropsis malorum* are a uniform light brown, without markings in the wall other than very small lighter spots. Mature spores of *Diplodia natalensis,* on the other hand, are a dark greenish brown, and the wall appears to be furrowed, or ridged lengthwise. This furrowing appears in many of the published figures of spores of this species.

THE PERFECT STAGE OF DIPLODIA NATALENSIS ON CITRUS

As stated above, the perfect stage of *Sphaeropsis malorum,* which the writers call *Physalospora malorum,* has been twice collected on citrus, once by Fulton in Baldwin County, Alabama, December, 1923, and once by Stevens at Satsuma, Florida, March, 1924. In the material secured at Satsuma, and on the same twigs with *Physalospora malorum,* was a Physalospora from which typical *Diplodia natalensis* spores were produced in pure culture. The cultures from single ascospores of this fungus and subcultures from the resultant pycnospores agreed in all respects with the citrus end-rot Diplodia. They grew readily at 36° C., produced the typical coloration on potato dextrose agar at these temperatures, produced the large mycelial masses on cornmeal agar flasks before fruiting, and the spores agreed in all respects with those of *Diplodia natalensis* from decayed citrus fruits.

So similar, however, are the perithecia and ascospores from which the spores of the Diplodia type were produced to those from which the spores of the Sphaeropsis type were produced that with the very limited material available the writers are at present unable to distinguish any differences. Indeed, since both occur on the same twigs and it is impracticable to make cultures from a given perithecium and then section it for microscopic study, it is not possible to say with certainty which form is in any particular section.

The perfect stage of *Diplodia natalensis* is apparently rather rare on citrus. In the course of our collections in Florida, the perfect stage has been found only once. We have also one specimen of *Physalospora* on citrus secured by Shear (No. 2892) in the Isle of Pines, in 1916. This is presumably, though by no means certainly, the perfect stage of *D. natalensis.* In this specimen the ascospores are slightly larger than those of typical *Physalospora malorum.*

In view of our admitted inability to distinguish between the perfect stages of these fungi, the possibility at once suggests itself that what we have here distinguished as *Diplodia natalensis* and *Sphaeropsis malorum* are really but varieties of a single species. Of this the writers can find no evidence whatever. We have had a large number of cultures of these two fungi under observation during the past two years; and not only do both show remarkable constancy in their various cultural and other characters, but no spore of the Sphaeropsis type has yet been found in cultures of *Diplodia natalensis,* nor has the reverse situation occurred.

It seems much more probable that we are dealing with two closely related species, the perfect stages of which are very similar indeed. That constant differences in the morphology of the perfect stages might be discovered if abundant ascospore material of *Diplodia natalensis* were available also seems probable. For the present the wisest course seems to be to consider the two fungi as separate species without attempting to assign a specific name to the perfect stage of *Diplodia natalensis.*

SUMMARY

The name *Diplodia natalensis* is here used to indicate the Diplodia which causes a stem-end rot of citrus fruit in Florida. The name *Sphaeropsis malorum* is used to indicate the common black rot fungus of apples in the eastern United States.

The perfect stage of *Sphaeropsis malorum* (*Physalospora malorum*) has been found twice on citrus. The perfect stage of *Diplodia natalensis* is a Physalospora very similar to *Physalospora malorum.*

Cultures of *Diplodia natalensis* can be distinguished from those of *Sphaeropsis malorum* by their superficial appearance at the time pycnospores are produced. The pycnidia of *Diplodia natalensis* are borne in "sclorotium-like masses" above the surface of the medium. The pycnidia of *Sphaeropsis malorum* are only very slightly raised above the surface of the culture medium.

Diplodia natalensis grows somewhat in culture at 37° C., whereas *Sphaeropsis malorum* grows slowly at 31° C., and very little above this temperature.

Pycnospores of *Diplodia natalensis* can be distinguished from those of *Sphaeropsis malorum* by differences in size and shape, in degree of septation, in color of the nature spores, and by the frequency with which hyaline spores are found. The pycnospores of *Diplodia natalensis* are somewhat wider than those of *Sphaeropsis malorum.* In general, spores of *Diplodia natalensis* remain hyaline until they are discharged from the pycnidium, and may be found in almost any preparation, whereas hyaline spores of *S. malorum* are comparatively rare. Pycnospores of *D. natalensis* are usually septate as soon as the spore wall becomes colored. Septate spores of *S. malorum* are decidedly less common than those with no septum.

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EXPLANATION OF PLATE VIII

A.—Pycnospores of *Sphaeropsis malorum* in culture from single ascospores from Citrus. ×

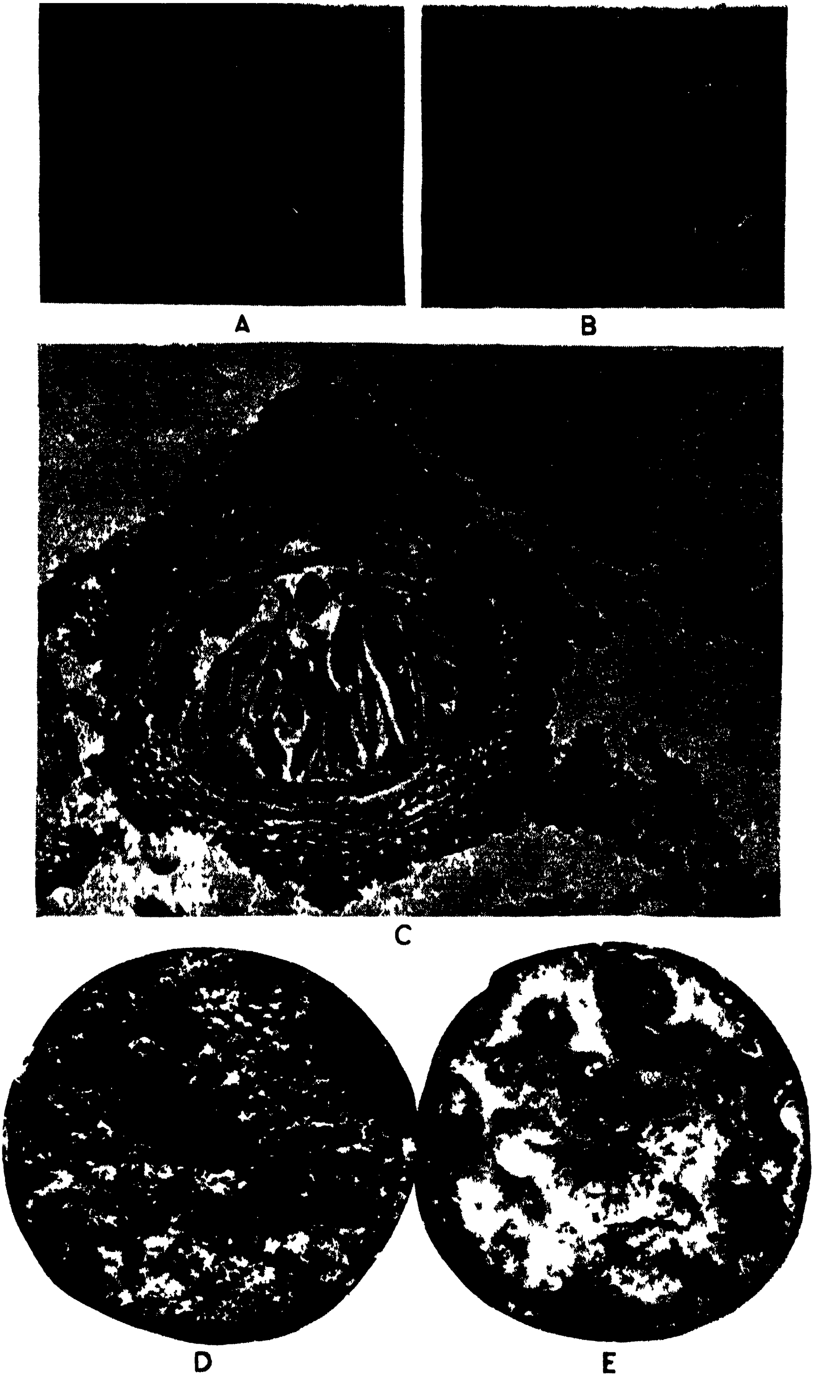
B.—Pycnospores of *Diplodia natalensis* in culture from single ascospores from Citrus. ×

C.—Longitudinal section of perithecium of *Physalospora* sp. on Citrus. ×

D.—Fruiting culture of *Sphaeropsis malorum* on cornmeal in flask, two months old, grown in green-house.

E.—Fruiting culture of *Diplodia natalensis* on cornmeal in flask, two months old, grown in green-house.

PLATE VIII



THE INHERITANCE OF DISEASE RESISTANCE IN WHEAT AND OATS[1]

E. F. GAINES

INTRODUCTION

It has been known since ancient times that plants and animals, including man, vary greatly in their ability to resist disease. Some races are more resistant to a given disease than others. Certain families within a race are more resistant than the average, and certain individuals within such families may be still more resistant.

More recent studies on the life histories of the causal organisms show that pathogenic fungi, bacteria, and infusoria are more or less restricted in their choice of host. There must be a rather delicate chemo-tactic balance between host and parasite in their growth relationships before infection resulting in disease can take place. This balance in certain cases is easily upset. For example, hogs may be vaccinated for cholera and thus be speedily changed from susceptible to immune individuals. Similarly, man may be immunized for life against smallpox or yellow fever by living through one attack of the disease. The substances in the blood that immunize against either pneumonia or tetanus, on the other hand, are cast off after a time so that the individual again becomes susceptible and may suffer a second attack as severe as, or worse than, the first.

This type of acquired resistance or induced immunity is not inherited in the ordinary sense, but each new generation must be vaccinated or inoculated with the proper virus in order to acquire immunity. Inherent or natural resistance appearing in families or races, if continued from generation to generation in matings among themselves, might be expected to be inherited in hybrid offspring when crossed with a susceptible race, like morphological characters. The easiest way to find out whether this is true or not would be to cross a resistant or immune individual on a susceptible individual and analyze the offspring in later generations. Considerable breeding work has been done along this line, and various investigators have published their results.

Biffen (3), in England, about 20 years ago crossed resistant and susceptible wheats in respect to stripe rust and found in the F_2 generation susceptible and resistant plants occurring in the ratio of 3:1. He selected the resistant recessives, some of which have continued to be resistant through all generations to the present day. He found resistance to mildew of barley inherited in much the same way. Nilsson-Ehle (9), in Sweden, found resistance to stripe rust in wheat inherited somewhat differently in his hybrids. When he crossed a wheat which was fairly resistant with one moderately susceptible, he obtained offspring more resistant than the resistant parent and more susceptible than the susceptible parent. This he thought was due to modifying multiple factors.

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Hayes, Parker, and Kurtzweil (6), in America, found resistance to stem rust of durum wheat was inherited when durums were crossed with the susceptible common wheats, but the resistance was usually associated with other durum characters, although they obtained one strain that was very resistant but in other respects resembled the susceptible common wheat ancestor.

Aamodt (1) demonstrated the necessity of using known biologic forms of stem rust in genetic studies of resistance in wheat. In an F_2 generation of Kanred and Marquis all the plants were susceptible when inoculated with a mixture of biologic forms, but F_3 selections inoculated with a pure culture of Form I gave results indicating a 1:2:1 segregation of immune, mixed, and susceptible families. He also found one family of spring type that was immune from the eight biologic forms from which Kanred is immune although Marquis is susceptible to six of them. Thus the resistance of Kanred was combined with the spring habit of Marquis.

At Cornell, McRostie (7) found two strains of bean anthracnose, each of which would attack and cause disease in certain varieties of beans but not in others. When a bean resistant to both strains was crossed on one susceptible to both, the F_2 offspring segregated in the ratio of nine resistant to seven susceptible plants. Either strain alone gave the expected ratio of 3 resistant to 1 susceptible. The record of the various types in later generations proved the correctness of his interpretation. Later (1921), McRostie (8) published an article in the Journal of the American Society of Agronomy in which he gave data to show that resistance to root rot and resistance to mosaic in beans were also inherited in his crosses. Many of his F_2 selections were shown to be accurately analyzed by their performance in the F_3 generation.

Barney (2), also of Cornell, recently analyzed three different typical crosses of oats in respect to their resistance and susceptibility to loose smut in which widely different ratios of resistance were obtained. He suggested the possibility that 1, 2, and 3 dominant factors for resistance would best explain the results.

Resistance of wheat to bunt and oats to covered smut has been studied in considerable detail at the Washington State College, two articles (4, 5) having been published on the inheritance of wheat resistance. Perhaps more data on the genetics of resistance to these two smuts have been obtained than at any other station. It is the purpose of this paper to present a summary of these data.

RESISTANCE IN WHEAT

Work on the resistance of wheat to bunt, or stinking smut, as it is commonly called, was begun at the Washington Experiment Station at Pullman in 1914. During the past ten years, considerable information has been obtained on the comparative resistance of many varieties as well as the mode of inheritance in 26 different crosses.

The bread wheats of all countries are found to be generally susceptible. When inoculated seed is sown under optimum conditions for infection, from 75 to 99 per cent of bunted heads is obtained. In testing some 500 varieties, however, a very few are found that are consistently resistant, producing less than 10 per cent. Just why it is rarely possible to get 100 per cent of bunted heads in the susceptible varieties is not known. Evidently certain plants escape infection, for when seed of such plants is sown under conditions favoring maximum infection, the resulting crop will be fully as susceptible as the unselected seed. The average susceptibility of 10 different pure lines has not been changed by selection, although bunt-free plants from badly infected plots have been selected year after year. Each variety has a rather constant index of susceptibility that fluctuates with environmental conditions but maintains its relative place in respect to other varieties. For example, Turkey produces from 1 to 15 per cent of bunt under the same conditions that Hybrid 128 produces from 75 to 100 per cent.

In 1918 the mode of inheritance of resistance was established for 2 crosses in which the resistant Turkey was the pollen parent while the susceptible Hybrid 128 and the resistant Florence were, respectively, the pistillate parents. The F_3 generation segregated into resistant, intermediate and susceptible types, some of which bred true in the F_4 in 1919. Transgressive segregates in the Turkey-Florence cross were obtained, that is, some were more resistant and some more susceptible than the parents. Data on selections of these two crosses from the fifth to the ninth generation have been obtained since 1918 and in addition, 19 other crosses have been investigated in the F_3 and later generations in respect to bunt resistance. From these data, it seems that Hybrid 128, Winter Fife and Velvet Node have no heritable factors for resistance to bunt; Fortyfold and Red Russian each have weak resistances which reduce the amount of bunt from 10 to 20 per cent; Marquis is resistant when sown in the spring but susceptible when sown in the fall. Turkey, Alaska and Florence each have differing concentrated resistances which reduce the amount of bunt 70 to 75 per cent compared with the susceptible varieties. These concentrated resistances are cumulative in effect when brought together by crossing, the resulting descendants segregating into immune, very resistant, and various stages of more or less resistant and completely susceptible classes. Martin, Hussar and White Odessa are bunt-free and are considered immune.

Martin has been under observation for seven years. Hussar has been bunt-free for four years under conditions favoring maximum infection. White Odessa was immune in 1921, 1922, and 1924, but in 1923 one smutted plant was found (probably a mixture).

In two crosses of susceptible varieties, Hybrid 128 × Marquis and Hybrid 128 × Velvet Node, only susceptible offspring were produced in subsequent generations. When Fortyfold and Red Russian, both intermediate for resistance, were crossed, intermediate and susceptible offspring were produced. Since both parents are considered intermediate, it would be expected that segregates would occur more resistant and more susceptible than the parents unless their feeble resistances were identical. Although several hundred third-generation families were tested, they were all as susceptible as the parents or more so. When the resistant varieties Turkey, Alaska, and Florence were crossed with the susceptible varieties Jenkin, Hybrid 128, and Jones Fife, the resulting offspring were more often susceptible than otherwise; in fact, the full resistance of the resistant parents was recovered in less than 2 per cent of the third generation families. When the same susceptible varieties were crossed with the immune Martin, Hussar, or White Odessa, there seemed to be a dominance of resistance, more than half of the resulting hybrids in the third and subsequent generations producing less than 5 per cent of bunted heads, about 20 per cent of which were bunt free. When resistant and immune varieties were crossed, an occasional segregate would show more susceptibility than the weaker parent, but on the whole the number of immune and highly resistant families was much larger than when immune and susceptible varieties were crossed. No doubt, the immune hybrids differed considerably in their potential immunity, but to prove this requires time and patience. There are two methods, however, by which this can be done: one is to back-cross them with susceptible varieties and then test the relative susceptibility of the offspring. The other is to test their vegetative vigor and yielding capacity when inoculated seed is sown under conditions favoring maximum infection in comparison with uninfected seed. Such a yield test was made in 1924 with 10 resistant or immune varieties. Some of the varieties were apparently reduced in yield by inoculating the seed while others were not. Martin and Hussar produced slightly more grain in one series of plats in which the seed was inoculated with bunt spores, than in the control where the seed was treated with copper carbonate. In another series, the smutted seed produced a smaller crop. Additional work along this line is necessary before definite recommendations regarding the elimination of seed treatment in commercial practice is safe. At any rate the immune wheats are more prepotent in transmitting resistance than those that show a slight infection. The word "prepotent" has been used for lack of a better term, although a more exact word would be preferable if one could be found. Probably what actually happens is the coming together of a greater number of unit factors for resistance, the cumulative effect of which is to make the total result appear as a dominant. A lesser number would give the recessive effect. Mendelian terminology is scarcely adequate to describe the inheritance of fluctuating quantitative characteristics like resistance to bunt, although there is little doubt that varietal differences in this respect are due to the cumulative effect of unit factors, and can be fixed in hybrid offspring like other factors, according to the known laws of heredity. It seems logical to assume that in time commercially desirable varieties immune from bunt will be extensively grown, thus eliminating the losses now caused by bunt. In fact a beginning has already been made. More than 5,000 acres were sown in the fall of 1924 to two of our immune hybrids, Ridit and Selection C.

Whether the development of varieties of other crops immune from their major diseases holds out unlimited possibilities might be a subject for controversy. But, certainly, enough now is known about variation in susceptibility and inheritance of disease resistance to warrant more experimentation along this line.

RESISTANCE IN OATS

The results with resistance to bunt in wheat have encouraged us to undertake similar work with resistance to covered smuts in oats. Covered smut is perhaps, next to bunt, the most noticeable and destructive cereal parasite of Eastern Washington. Some 200 varieties tested show a range from complete susceptibility to immunity. The common commercial oat varieties of the Northwest are generally susceptible, but a much smaller percentage of infection occurs when the seed is inoculated with smut spores than is obtained with bunt in wheat, because of the protective covering of the seed. When the hull is peeled off and the naked kernel blackened with spores, from 75 to 100 per cent of smutted plants are produced.

In 1914 a variety of Red Rustproof by the name of Texas Red was brought back from the Sixth National Corn Show at Dallas, Texas. This Red Rustproof selection has been immune to covered smut in all tests, even when the hull was removed and the naked seeds blackened with viable spores before sowing.

Red Rustproof can be crossed with the other species of oats, although many sterile flowers are found in the F_1. Enough seed is produced, however, to give fairly large populations in the F_2 and later generations. Four crosses with this variety have been analyzed in the F_3 with respect to resistance to covered smut as well as selections in later generations. In 1919 the third generation of Red Rustproof and Black Tartarian was grown in the cereal nursery. As there were several other segregating factors of genetic interest, the analysis of the 117 F_3 families was turned over to one of our graduate students, Steich Wakabayashi. Mr. Wakabayashi analyzed the material in considerable detail, a summarized report of which he published in the Journal of the American Society of Agronomy, October, 1921.

In 1922 the analysis of an F_3 of Red Rustproof and Abundance was undertaken by E. F. Landerholm as major research work in his senior year at the State College of Washington. The compilation of the data proved too great a task, as there were 345 F_3 families, and the completion of the work was delegated to others after Mr. Landerholm graduated.

In 1923 the data on 150 F_3 families of a cross between Red Rustproof (C. I. 2140) and Large Hulless (C. I. 278) were taken by Elmer Ausemus, and this material, together with a summary of the other two crosses, was presented as his Master's Thesis in June, 1924.

Black Tartarian (Wn. 762), Abundance (C. I. 1192), and Large Hulless (C. I. 278) represent three different species and are very unlike taxonomically, but they are more closely related to each other genetically than to Red Rustproof. Large Hulless is extremely susceptible, often producing more than 90 per cent of smutted panicles. Black Tartarian and Abundance are more resistant, producing only 20 to 30 per cent of smutted panicles when sown under conditions favoring maximum infection. This is partly due to the protective effect of the adhering lemma, but when this is removed they seldom produce more than 75 to 85 per cent of covered smut.

The inheritance of the immunity of Red Rustproof is essentially similar in all three crosses. It is preponderantly dominant. In only five of the 612 F_3 families tested was the susceptibility of the weak parent recovered. In the cross with Black Tartarian, only 10 per cent of the F_3 families produced any smut at all. The smuttiest F_3 family produced but 15 per cent of smut compared with 34 per cent for the Black Tartarian check rows. In the cross with Abundance, out of 345 F_3 families, only nine produced more than 5 per cent of smutted panicles, 50 others produced from a trace up to 5 per cent, and 286 were smut-free. In the cross with Large Hulless, 46 out of 149 F_3 families produced traces of covered smut. No smut was found in 103 of the rows, seven of which were homozygous for the hulless character, and five of which produced immune offspring in the fourth generation. These seven hulless rows upset the commonly accepted view that the hulless varieties as a class are very susceptible to covered smut. The check rows of Large Hulless produced 86 per cent of smutted panicles, but the smuttiest F_3 row produced only 51 per cent. Only 16 F_3 rows produced more than 10 per cent.

The 496 immune F_3 families of these three crosses no doubt vary greatly in their potential immunity, but as yet no adequate method has been developed for determining this point. Back-crossing with susceptible varieties, as was suggested for the immune wheats, should in time bring out these differences, but it is such a slow and expensive process as to be impracticable for more than a very few selections.

In so far as the data have been analyzed, immunity does not seem to be linked with any of the outstanding characteristics of Red Rustproof. Hulless segregates, white and black hulled segregates, and F_3 families with side type of panicle have been found which are immune. Likewise, some of the susceptible segregates have the red lemmas, hairy base, and bearded secondary kernels of Red Rustproof.

The dominance or "prepotency" of this immunity to covered smut in oats could be explained on the basis of multiple factors which are cumulative in effect. More factors would need to be assumed, however, than have been assumed in the immunity work with wheat or else a larger effect of each individual factor would have to be assumed. Our knowledge of comparative susceptibility of standard oat varieties leads us to believe that there are several factors, for the different varieties vary all the way from complete immunity to complete susceptibility. A definite analysis of the characters just described would be premature until more work has been done. The principle of resistance heritability in oats, however, is considered established. This principle is gaining wider credence for other diseases, as plant breeders throughout the country investigate the subject genetically. Parker (10), of the Kansas Agricultural Experiment Station, has shown that resistance to crown rust of oats is recessive, but caused by multiple factors. Barney (2), of Cornell, working with loose smut in oats, on the other hand, found resistance dominant. This in general agrees with our covered-smut results. It may be, as Reed (11) has suggested, that the two smuts of oats react similarly to a given host variety in respect to resistance.

DISCUSSION AND CONCLUSIONS

In addition to the growing volume of literature on the inheritance of resistance of plants to disease, there are a few notable examples of inherited resistance to insect attack. The vineyards of Europe have been replanted during the past 30 years with hybrid stocks from American species resistant to the plant louse, Phylloxera, a root insect that for a time threatened the grape industry of the world. Recently investigators have begun fighting the chinchbug with resistant varieties of corn.

Although little has been published on the subject, inherited resistance in the animal kingdom is probably similar to that in the plant kingdom. In work with mice it has been found that the inheritance of immunity to tumor is dominant, but caused by multiple factors.

In view of the ever-increasing positive evidence of the general application of the principle of genetics in developing economic forms of life resistant to their major diseases, it seems that investigators generally would be warranted in giving more attention to this phase of research as a possible solution of the major diseases of economic importance. Is it too much to assume that an apple variety may be found which the Codling Moth would not infest, or that a tree may be found that would not act as host to San Jose scale? Might not a pig be found naturally and genetically immune from hog cholera?

A potato resistant to its destructive major diseases would be of interest and value. The forage crops offer great possibilities. One has but to mention the rusts, mildews, root-rots, bacterial blights, infusorial troubles, and insect pests, to excite the imagination of the geneticist or selectionist. Any form of life possessing inherent capacity to fight its enemies through the chemo-tactic reactions of its germ-plasm has a great advantage over its fellows. Other characteristics being equal, it would be much preferred. The economy saved in spraying and seed treatment, in addition to actual loss on account of the disease, would commend it at once.

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QUANTITATIVE DETERMINATION OF SULFUR FUNGICIDES ON FOLIAGE

H. W. FITCH

The persistence of sulfur fungicides on the fruit and foliage of trees is an important factor to be considered in protecting against infection by pathogenes. General experience with the use of sulfur materials applied as a protectant suggests a progressive decrease in the amount per unit area with the passing of time. Assuming uniform distribution at the time of application, fungicidal effectiveness, below the optimum, is proportional to the relative amounts of sulfur residue on the foliage. Direct decrease is brought about by weathering factors, such as washing by rain or dew, mechanical action of wind, and slow chemical change in the exposed spray coating. An indirect decrease in effectiveness results from the growth extension of plant parts, either by the enlargement of organs or by the development of new organs subsequent to the application.

In practice, the interval between applications should depend on the rate of weathering and on growth extension. The latter can be judged by careful observation. So far as the former is concerned, observations are often misleading. Present-day spray calendars are attempts to indicate critical periods, which, while based on results of investigations, are at best only approximations. In any particular season, section, variety, or planting, the actual times and numbers of applications for adequate protection may vary widely from those indicated in the standard spray calendars. The complicated set of local factors makes difficult the determination of the effective period for any given application. A reliable chemical test for determining the amount of the fungicide present per unit area would seem to be a desirable aid in forming a judgment as to the proper time for renewal. Such a test should be simple, rapid, and reasonably accurate. Especially refined methods of chemical analysis, while more accurate, would not well serve the practical purpose, because of requirements in professional training, apparatus, and time.

While conducting fruit dusting investigations during the summer of 1921, the writer made numerous tests to determine the persistence of copper dust coatings on apple foliage, using the method employed by Winston and Fulton.[1] The desirability of making tests for the determination of the persistence of sulfur was very apparent. Therefore, during the winter of 1922, the following method was developed and has been tested under field conditions during the seasons of 1922, 1923, and 1924.

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[1] Winston, J. R., and H. R. Fulton. The field testing of copper spray coatings. U. S. Dept. Agr. Bul. 785: 1–9. 1919.

METHOD[2]

A condensed statement of the successive steps to be taken in making the test may be outlined as follows:

1. Collect at least three representative samples of forty leaves each.
2. Make separate determinations for each sample as follows:

(a) Place 125 cubic centimeters of carbon tetrachloride (C. P.) in a 600 cubic centimeter beaker.

(b) Immerse the leaves in the carbon tetrachloride for 3 or 4 minutes and stir occasionally.

(c) Remove leaves from wash.

(d) Heat wash slowly until it just starts to boil.

(e) Filter into 150 cubic centimeter flask.

(f) Evaporate filtrate down to 20 cubic centimeters.

(g) Pour hot filtrate into a thin watch glass of known weight and set aside until the carbon tetrachloride is entirely evaporated.

(h) Determine weight of sulfur residue present.

The writer has found forty leaves of fairly uniform size to constitute a satisfactory sample for making this test. By the use of a planimeter, it was found that forty apple leaves had an average total area of 186.7 square inches. If one desires, he may secure a uniform surface area for testing by measuring each sample. Green weight determinations can not be depended upon, because there is considerable variation in thickness and consequently in the weight of different leaves. In making a series of successive tests during a season, it is desirable to take the three samples, one from each of three trees, and to take samples for each successive test from the same trees. Care should be exercised in collecting these samples in order to prevent undue rubbing of the leaves, which would remove the sulfur.

The samples should be tested shortly after collection, since drying interferes with the determination. Instead of immersing the leaves, a stream of carbon tetrachloride may be directed against the surfaces from a wash bottle.

When the wash is heated, all of the sulfur goes into solution and so will pass through filter paper. It is necessary to use a high grade of filter paper to eliminate soil particles and other foreign matter. Half or more of the solvent may be recovered by condensing the vapors.

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[2] Credit is due Prof. L. J. Cross, New York State Chemist, for assistance in formulating this test.

At the beginning of a series of tests, a 125 cubic centimeter sample of carbon tetrachloride should be run through the procedure to test the purity of the chemical. In order to correct an error that may have occurred from substances other than sulfur appearing in the filtrate, it is desirable to test an equal number of leaves that have not received an application of sulfur. If a residue is found either in the tetrachloride or from non-sprayed or dusted leaves, it should be figured as a correction in the determinations. Satisfactory indications of the loss of the sulfur from the leaves can ordinarily be obtained by making collections and tests of foliage samples every four days.

This test was designed particularly for testing the persistence of sulfur on apple foliage, but it has also been used by the writer for testing sulfur persistence on the fruit of apple and the leaves of peach, cherry, plum, prune, quince; and by A. L. Pierstorff on currant, gooseberry, and roses.

DISCUSSION OF RESULTS

When made at various periods following an application of dusts or sprays throughout the season, the tests indicate a gradual decrease in the amount of sulfur present on the foliage. During the first few days after an application, the loss of sulfur from the foliage was often rather rapid, particularly when dust was used.

The field tests of expanded leaves indicate that from 67 to 86 per cent of the sulfur applied in the form of lime sulfur solution actually adheres to the foliage of sprayed trees at the time of application, while 36 to 66 per cent of the sulfur used is to be found on dusted foliage immediately following treatment. These variations in percentages depend on the efficiency of machines, thoroughness of application, and conditions which prevail at the time the materials are applied. Strong wind is found to greatly decrease the amount of materials adhering to the foliage in either case. Excessive quantities of spray, beyond a certain limit, are of no value since the leaves retain but a certain maximum of the liquid. This is also true of the dusts, of which only a certain maximum can be made to adhere to the foliage. Trees with heavy foliage catch a greater proportion of the materials used than open trees.

The amount of sulfur residue necessary for adequate protection against any specific disease will doubtless vary with a complex set of factors. There is clearly a limit at or above which, with adequate distribution, protection will be secured and below which protection will be increasingly insufficient. The exact determination of this limit would necessitate experiments covering a period of years. The best practice should aim at keeping the protective sulfur coating safely above the minimum.

Table 1 shows the results of sulfur persistency tests conducted on sixteen years old Greening apple trees. For this test the sprayed trees received per tree four gallons of 1 to 40 liquid lime sulfur, and the dusted trees received one and one-half pounds of a 90–10 sulfur lead dust.

TABLE 1.—*Results of tests to determine the persistency of sulfur on apple trees*

Date of test	Spray test (Milligrams*)	Dust test (Milligrams*)	Rainfall (Inches)
Day of treatment	60	260	.00
2 days later	50	185	.05
6 days later	50	100	.70
10 days later	50	60	1.93
14 days later	40	45	.07
18 days later	40	45	.00
22 days later	40	40	.00
26 days later	25	30	.00
30 days later	20	25	.00

* *i.e.*, Milligrams sulfur per sample of 40 leaves.

A comparison of data obtained from these tests with infections occurring during the season indicates that at least 30 to 40 milligrams of sulfur should be present on forty leaves, or 186.7 square inches of leaf area, to afford adequate protection. When a test shows such a small amount of the protective sulfur coating to be present, it is certainly approaching the minimum limit, and the coating should be renewed before the next infection period. Under ordinary weather conditions the progressive decrease in the protective coatings will usually reach the above suggested minimum for protection in about three weeks, and it cannot be depended upon for a longer time. As decrease results both from weathering and growth extension, the fruit grower should watch the rate of growth and the weather very carefully during critical periods and be prepared to spray or dust before a storm period occurring at any time within 2 weeks after the last application. These sulfur tests indicate that, for adequate protection, the time interval between applications of dust does not differ materially from that between spray applications. When the sulfur coating on either dusted or sprayed apple trees was kept at 40 milligrams, or above, per standard sample during the season, 90 per cent or more of scab-free fruit was produced.

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PHYTOPATHOLOGICAL NOTES

Summer Meeting of the American Phytopathological Society and Annual Meeting of its Pacific Division. C. E. Owens, Secretary-Treasurer of the Pacific Division, under date of March 19 writes that the probable date of their annual meeting, and therefore the summer meeting of the Society which will be held in conjunction with it, has been set for June 22–24 at the Oregon Agricultural College, Corvallis, Oregon. The meeting will also be in conjunction with the Northwest Association of Horticulturists, Entomologists, and Plant Pathologists.

The Pacific Division of the American Association for the Advancement of Science will hold its meeting in Portland, Oregon, June 17–20. A field trip, taking in the Mt. Hood Loop and stopping at Hood River for the night, has been planned for Saturday, June 30, for persons attending the Portland meeting. The parties attending the Portland meeting can take this in and proceed from Hood River to Corvallis on Sunday for the meetings there Monday, Tuesday, and Wednesday, June 22–24. There is a paved highway the entire distance from Hood River to Corvallis. Professor Owens is hoping for a large representative attendance from the East and would like to know as far in advance as possible who will attend. All members expecting to be present at the Corvallis meeting should notify Professor Owens at an early date.

Summer Field Meeting of Cereal Pathologists. A meeting of cereal pathologists will be held from July 8 to July 14, at Ames, Ia.; St. Paul, Minn.; and Fargo, N. D. The tentative program is as follows:

July 8 Meet at Ames.
July 9 Field work between Ames and Albert Lea, Minn.
July 10 Field work between Albert Lea and St. Paul, Minn.
July 11 Inspection of field plots, field trip, St. Paul.
July 12 Rest if desired. Evening train to Fargo, N. D.
July 13 Meet at Fargo.
July 14 Field trips in vicinity of Fargo.

The committee on arrangements consists of the following: I. E. Melhus, H. B. Humphrey, H. L. Bolley, and E. C. Stakman.

Personals. Dr. George K. K. Link has been appointed Associate Professor in charge of the newly created Division of Plant Pathology of the Botany Department in the University of Chicago.

Dr. H. A. Edson has recently been promoted to the position vacated by Dr. W. A. Orton as senior pathologist in charge of the Office of Cotton, Truck, and Forage Crop Disease Investigations, U. S. Department of Agriculture.

Dr. G. H. Coons, pathologist of the Michigan station, has been granted a year's leave of absence in order to undertake studies of diseases of sugar beets in the Office of Sugar Plant Investigations, U. S. Department [illegible] Agriculture.

Dr. N. J. Giddings, head of the Department of Plant Pathology [illegible] the West Virginia State College of Agriculture, at Morgantown, W. Va., and Dr. L. E. Melchers, head of the Department of Botany and Plant Pathology of the Kansas State College of Agriculture, Manhattan, Kansas, spent the month of January in Washington, D. C., compiling the annual summaries on the occurrence of diseases of fruit and cereal crops, respectively, for the Plant Disease Survey.

Dr. P. J. Anderson, pathologist of the Massachusetts Experiment Station, has resigned his position to become Director of the Connecticut Tobacco Experiment Station at Windsor, Conn., a branch of the Conn. Agricultural Experiment Station. He will be succeeded at the Massachusetts station by W. L. Doran, who has been in charge of vegetable pathology at the Massachusetts market garden station at Waltham.

J. L. Seal, formerly of the Department of Plant Pathology, University Farm, St. Paul, accepted a position, beginning January 1, 1925, with the Florida Agricultural Experiment Station. His headquarters for the present are at Miami, Florida.

Dr. James R. Weir, pathologist in charge of Pathological Collections, Bureau of Plant Industry, has completed a two-months' period of service with the Tropical Plant Research Foundation on a survey of sugar cane fungi in Cuba and afterwards collected and studied the diseases of tropical plants in Haiti, the Dominican Republic, and Puerto Rico.

Mr. John R. Winston, Associate Pathologist in the Office of Fruit Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, who has been in charge of the U. S. Citrus Disease Field Laboratory at Orlando, Fla., since 1916, engaged in citrus disease work, has resigned to accept a position as Pathologist with the Peninsular Chemical Company, Orlando, Florida.

Mr. D. M. Weller, a student of Dr. W. J. G. Land, of the University of Chicago, has joined the staff of the Pathology Department of the Experiment Station of the Hawaiian Sugar Planters' Association. The staff of the Department at the present time is as follows: H. Atherton Lee, Pathologist; J. P. Martin and Clyde C. Barnum, Assistant Pathologists; D. M. Weller, Histologist; and Z. A. Romero, Photographer. This Pathology Department is probably unique in that its attention is devoted solely to a single crop, sugar cane, and on this crop solely to the limited area of the four principal islands of the Hawaiian group.

PHYTOPATHOLOGY

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THE TRANSMISSIBLE LYTIC PRINCIPLE (BACTERIOPHAGE) IN RELATION TO PLANT PATHOGENES

G. H. Coons and J. E. Kotila

With Plates IX to XII

The work of d'Herelle (1) with various animal pathogenes, especially *Bacillus dysenteriae* Shiga, has established the existence of a transmissible lytic principle[1] effective against certain bacterial strains. d'Herelle has named the principle "the bacteriophage," and he believes his experiments show the existence of an ultra-microscopic parasite, *Bacteriophagum intestinale* d'Herelle, (3) living as an obligate parasite on bacteria. The phenomenon of lysis of bacterial cultures into which the active filtrate has been introduced is explained as due to a single species of organism capable of growth and capable of attacking a wide range of bacterial species. Material investigated by him has always been derived from the intestinal tracts of animals, and the lines of investigation have proceeded upon the assumption of an animal source of the lytic principle. The organisms used have been animal pathogenes.

The work of d'Herelle has stimulated a large amount of study of this phenomenon, and many organisms pathogenic to man and lower animals have been studied in this regard. Very little has as yet been published on the relation of the transmissible lytic principle to the bacterial pathogenes of plants.

Gerretsen and Sack, and Söhngen and Gryns (4), both groups working independently at first and later in cooperation, probably were the first to record the d'Herelle phenomenon in relation to bacteria associated with plants. These investigators were successful in isolating lytic principles from nodules of various leguminous plants. These were specific for the bacteria of the legume concerned. Isolations of the lytic principles were also made from roots and stems, but not from leaves of the legumes; from garden and field soil, but not from heath or forest soil. The lytic principles were found to withstand desiccation, as well as heating at from 60° to 65° C. for fifteen minutes; and they were at least eight times as resistant to ultra-violet light as were the bacteria upon which they were effective.

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[1] In this article the terms commonly used in the literature with reference to the d'Herelle phenomenon have been employed. The writers feel that their experiments have not been of the type to bear directly on the moot point of whether lysis is brought about by a living ultra-microscopic organism or whether some other explanation is more tenable. The term "transmissible lytic principle" has been employed following common usage in this country (2).

Independently, Mallmann and Hemstreet (5) also demonstrated the d'Herelle phenomenon, using an organism obtained from a plant source. These workers obtained what they call an "inhibitory substance" from cabbage which had been rotted by fluorescent organisms. They were able to demonstrate marked inhibition of growth with extremely high dilutions of the filtrate, but were not able to demonstrate actual lysis. They present evidence of the increase of activity upon successive transplantings and filtrations and they obtained inhibition with a dilution of 1 : 100,000,000,000. The bacterial organism isolated by Mallmann and Hemstreet produced a slow rotting of cabbage, several weeks being required for complete liquefaction. It is stated by them that the organism belongs to the fluorescent group rather than to *Bacillus carotovorus* and its allies. These workers further tested the "inhibitory substance" against known plant pathogenes, *Bacillus carotovorus*, Spieckerman's bacillus, and the "potato rot bacillus"; and with the first two, except where large amounts of filtrate were used, no inhibition was noted. The "potato rot bacillus" was not inhibited at all.

The work of Mallmann and Hemstreet very evidently opens up a very interesting field of experimentation from the point of view of plant pathology. In order to ascertain whether the d'Herelle phenomenon could be demonstrated with known plant pathogenes, such as *Bacillus carotovorus* Jones, *B. atrosepticus* vanHall, and *Bacterium tumefaciens* EFS. and Towns, and to determine the rôle played in pathogenesis, experiments were undertaken early in 1924. Following the methods of d'Herelle[2] an apparently sterile filtrate was obtained from a carrot which had decayed with typical soft rot when stored in sand in a basement. When various quantities of this filtrate (1 drop, 10 drops, 2 cc.) were placed in tubes containing suspensions of *Bacillus carotovorus* in alkaline Liebig beef extract broth, an inhibiting effect on the growth of the organism was noted after three days' incubation at 25° C. All tubes containing the filtrate were less turbid than the check.

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[2] A portion of a rotted carrot, and adhering sand, was incubated in 25 cc. of standard beef broth for 24 hours. The mixture was filtered through filter paper and then through filter paper lined with infusorial earth. The clear filtrate was then refiltered through a Pasteur bougie (L7) and an apparently sterile filtrate was obtained which was demonstrated to contain the transmissible lytic filtrate. Subsequent filtrates were obtained from pooled cultures of an earlier series of tests. Alkaline Liebig beef broth (pH 8) was used in all tests unless other medium is mentioned.

Similar results were obtained from filtrates obtained from river water and from soil which had been drenched with heavy suspensions of pure cultures of *B. atrosepticus*. This soil had been used in experiments to determine whether infested soil is a source of the black leg disease of potatoes (7).

INCREASE IN POTENCY OF SUCCESSIVE FILTRATES

The potency of the lytic principle was found to be increased after repeated association with susceptible organisms. The original material showed only inhibitory effects, whereas, after exposure to susceptible organisms and refiltering four times, the activity of the filtrate so obtained was so increased that it was effective in causing inhibition up to a dilution of 1:100,000,000 and capable of causing distinct clearing of suspensions in the lower dilutions. In the case of the filtrate of four passages the effect was apparent after fourteen hours, whereas the first filtrate did not show inhibition effects until after three days.

After the same material had undergone exposure to susceptible organisms and refiltering four times more, the two filtrates were compared by means of a dilution experiment. Two series of tubes, each containing 9 cc. of alkaline beef broth, were treated as follows: One cc. of the material of the fourth passage was added to the first tube of one series and was mixed thoroughly by shaking. A dilution of 1:10 was thus obtained. By means of another sterile pipette, 1 cc. of this dilution was added to the next tube of the series, making a dilution of 1:100. The process was repeated, using a fresh sterile pipette each time until a dilution of 1:100,000,000 was obtained. The second series was treated similarly, using at the start 1 cc. of the material of the eighth passage. Each tube was then inoculated with 1 cc. of a suspension of *B. carotovorus* made by diluting 5 cc. of a 3-day-old broth culture with 25 cc. of sterile broth. Two tubes of sterile broth were inoculated with the same quantity of organisms to serve as checks. The invigorating effects of the additional exposures to the susceptible organism can be seen from table 1.

It is seen from the above results that the effect of the material of the eighth passage is more pronounced than that of the fourth passage. The inhibition effects upon growth were evident earlier and lasted longer in the case of the eighth filtrate. The increase of potency of the eighth filtrate can be seen by comparing figures 1 and 2 of plate IX. The relative clear-

TABLE 1.—*Degree of lysis with Bacillus carotovorus: Comparison of the lytic principle of the 4th and 8th passage*

Times invigorated	Incubation period	1: 10	1: 100	1: 1000	Dilutions		1: 1 million	1:10 million	1: 100 million	1: 1000 million	check
					1: 10,000	1: 100,000					
4	1 day	+	+	++	+++	+++	+++	++	–	–	–
8	1 day	++++	++++	++++	++++	++++	++++	+++	+	–	–
4	2 day	+++	++	+++	+++	++	+	++	–	–	–
8	2 day	+++	+++	++++	++++	++++	++++	++	+++	–	–
4	3 day	++++	+++	+++	+++	++++	++++	++	–	–	–
8	3 day	++	+++	+++	+++	+++	++++	++	++++	–	–
4	4 day								+	+	–
8	4 day								+++	–	–
4	8 day								+	+	–
8	8 day								+++	+	–
4	14 day	+	+	+	+	+	+	+	+	+	–
8	14 day	++++	++++	++++	++++	++++	+++	++	+	+	–

Note: Readings on 4th and 8th days were made only for the highest dilutions.

– No inhibitory or lytic effect, medium very turbid.

+ Slight inhibition or lysis effect, medium turbid.

++ Medium inhibition or lysis, medium slightly turbid.

+++ Strong inhibition or lysis, medium very slightly turbid.

++++ Very strong inhibition or lysis, medium very clear.

ness of medium as shown in the photographs indicates that the inhibition to growth is not so strong in the tubes labelled "F 13" (four passages) as in the series of "F 23" (eight passages). The photographs were taken after an incubation period of three days.

MICROSCOPIC STUDY OF CULTURES

Examination of a great many hanging drop cultures made from normal cultures and those containing the lytic principle showed certain characteristic developments in the "phage" cultures. Decrease in motility, agglutination, and malformation of the organisms were constantly found in material from culture tubes to which the lytic principle had been added. The normal cultures did not show these peculiarities. These observations were made upon the three plant pathogenes already named. The shapes of malformed organisms from "phage" cultures varied considerably. Some individuals were much elongated, others were elongated and swollen, some were bulged at one end, while still others were short and markedly distended at the middle to a spindle shape. Enlarged spherical forms were observed also, but none of these were observed to burst as is reported by d'Herelle.

Some interesting observations were made in sets of hanging drop cultures which were made from a certain dilution experiment. In this experiment dilutions of 1:2,000, 1:20,000, 1:200,000, and 1:2,000,000 were made. The first set of hanging drop cultures was made from these dilutions immediately after the experiment was started, while the second set was made from the same tubes after four days incubation. Examination of the hanging drops of the first set after a period of twenty-four hours showed agglutination and malformation in the slides made from the 1:200, 1:2,000 and 1:20,000 dilutions. In the highest dilutions, 1:200,000 and 1:2,000,000, the organisms were actively motile and appeared no different from the check. The hanging drops of the second set were examined immediately after they were made and, in the case of the lower dilutions, were like the hanging drops of the lower dilutions of the first set. In the hanging drops of the two highest dilutions, however, agglutination was observed. The same set of slides (4-day) were again examined after twenty-four hours, and it was found that the organisms had become malformed, while the corresponding hanging drop cultures of the first set remained like the checks, apparently normal. In the meantime the test-tube cultures of the two highest dilutions had become much less turbid than the check, indicating the presence of the lytic principle even after high diluting. These observations seem to indicate that the lytic principle is corpuscular and increases in the course of a period of culture. It is plausible to explain these results by assuming that in the 1:200,000 and 1:2,000,000 dilutions few particles of the lytic principle were present at the beginning of the incubation period, and when hanging drops were made from these by means of a straight needle none of the particles happened to be carried over, hence the bacteria in these drops remained normal. However, after four days' incubation the number of the particles had increased to such an extent that they had got the upper hand, so to speak, of the bacteria, as evidenced by the agglutination observed, and on further incubation caused malformation and behavior typical of the d'Herelle phenomenon. The development of agglutination seems to presage the subsequent inhibition effects in the cultures.

TEMPERATURE RELATIONS

Work with the animal pathogenes carried on by other workers had been done usually at 37° C., which is near the optimum for the organisms used. The optimum of the organisms used in our tests is much lower. Preliminary experiments had shown a marked difference in the activity of the lytic principle between culture tubes incubated at 37° C. and room temperature. These led to further tests on the effects of various temperatures on the activity of the lytic principle. For this purpose an experiment was conducted using a nine-chamber differential thermostat in which the average temperature range was from 7.8° C. to 36.1° C. with a variation of not more than 1½ degrees. One broth culture containing the lytic principle and one check culture of *B. carotovorus* were placed in each chamber and incubated for six days with the following results (Table 2).

Very little difference was observed between the check and the "phage" tubes at the two highest and the two lowest temperatures. The checks showed a very poor growth at these temperatures, while at temperatures more nearly optimum for the organism the check tubes became turbid in two days while the "phage" tubes remained clear. Evidently conditions which are favorable for the growth of the organism likewise are essential for the development of strongest lysis.

SPECIFICITY OF THE LYTIC PRINCIPLE

d'Herelle has suggested that the bacteriophage is polyvalent and has postulated one organism which is capable of attacking all species of bacteria. Since the early work, strains of bacteriophage, in the terminology of d'Herelle, both polyvalent and univalent have been isolated by various workers. The isolation from carrot made by us was tested out against several plant and animal pathogenes.

TABLE 2.—*Effect of various temperatures on lytic action: Tests with Bacillus carotovorus; incubation period, six days*

No. of chamber	Average temp. C.	Results	
		Plus filtrate	Check
1	7.8	no growth	no growth
2	11.7	little growth	little growth
3	16.8	++++	-
4	19.5	++++	-
5	22.0	++++	-
6	23.4	++++	-
7	24.7	+++	-
8	26.7	++	little growth
9	36.1	(?)	no growth

−No lytic effect, medium very turbid.
+Slight lytic effect, medium turbid.
++Medium lytic effect, slightly turbid.
+++Strong lytic effect, very slightly turbid.
++++Very strong lytic effect, very clear.

Numerous tests of the lytic principles isolated by the writers against *Bacterium tumefaciens, Bacillus carotovorus,* and *B. atrosepticus* have shown that these are effective against all, but not equally. The lytic principle has seemed to be most effective against *Bact. tumefaciens* (from peach, obtained from Dr. Smith), followed in order by *Bacillus carotovorus* (Kotila isolation) and *B. atrosepticus* (Kotila isolation). Table 3 and plate X show the effect of the lytic principle isolated from carrot in various dilutions against these organisms. Although differences in effectiveness of the lytic principle were observed, no such marked specificity was apparent as noted by Gerretsen et al. (3).

A series of dilutions ranging from 1:10 to 1:100,000,000, using potato broth to which 1 per cent dextrose was added and whose reaction was neutral, showed lysis of *Bacillus carotovorus* in all except the greatest dilution. These results are at variance with reports in the literature, which have indicated that the presence of sugar prevented lysis.

In other tests standard beef broth has been used without the addition of the excess of sodium hydroxide. Comparative dilution tests have shown no marked difference in lysis between the ordinary standard beef broth and the strongly alkaline broth. This result also is at variance with some reports in the literature.

Apparently sterile broth containing the lytic principle (9th passage) was sealed in a flask with a paraffined stopper. After 5½ months it showed no evidence of contamination and it was again tested. The filtrate

TABLE 3.—*Dilution test with lytic principle: Fourth passage filtrate tested against 3 organisms; room temperature*

Organism	Interval	Dilutions								Check No lytic principle
		1:10	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	1:10 million	1:100 million	
Bacillus carotovorus (54)	17 hrs.	+++	+++	+++	+++	-	-	-	-	-
	41 hrs.	++++	++++	+++	+++	+++	+++	++++	+++	-
	3 da.	++++	++++	++++	++++	++++	++++	++++	+++	-
	5 da.	-*	-*	-*	-*	-*	-*	-*	-*	-
	10 da.	-	-	-	-	-	-	-	-	-
Bacillus atrosepticus (10)	17 hrs.	+++	+++	+++	-	-	-	-	-	-
	41 hrs.	++++	++++	++++	+++	+++	+++	++	+	-
	3 da.	+++	++++	++++	+++	+++	+++	+++	+++	-
	5 da.	+++	+++	++++	++++	++++	++++	+++	+++	-
	10 da.	-	-	-	-	+++	+++	++++	-	-
Bact. tumefaciens (146)	17 hrs.	++++	+++	+++	+++	-	-	-	-	-
	41 hrs.	++++	++++	++++	++++	++++	++++	+++	+++	-
	3 da.	++++	++++	++++	++++	++++	++++	+++	+++	-
	5 da.	-	++++	++++	++++	++++	++++	+++	+	-
	10 da.	-	++++	++++	++++	++++	++++	++++	++++	-

* Cloudy, but distinctly less than check; lower dilutions, showing slightly more than higher dilutions.

- No inhibitory or lytic effect, medium very turbid.
+ Slight inhibition or lysis effect, medium turbid.
++ Medium inhibition or lysis, medium slightly turbid.
+++ Strong inhibition or lysis, medium very slightly turbid.
++++ Very strong inhibition or lysis, medium very clear.

was not so active as when put aside but was capable of inhibiting growth. It was not capable of causing clearing except with *Bacillus carotovorus.*

In this test various other bacterial organisms were included, many representing organisms not previously used, and some representing other strains of those previously used in the tests. For comparison with the results of other workers, three animal pathogenes were included. The results of this test are given in table 4.

TABLE 4—*Test of lytic principle against various organisms: Filtrate (9th passage) used after 5½ mo. storage, 1 drop per tube*

Organism	Plus lytic principle 2-11-25	Plus lytic principle 2-13-25	Check 2-11-25	Check 2-13-25
Bacillus carotovorus (Bot. Exp. Strain)	+	+	—	—
B. carotovorus (Strain 54)	+++	++	—	—
B. atrosepticus "30"	—	+	—	—
Bact. pruni	—	—	—	—
Bacillus melonis	—	—	—	—
Bact. tumefaciens (from Hop; courtesy Dr. Smith)	—	—	—	—
*Bacillus amylovorus**	—	—	—	—
B. typhosus	—	±	—	—
B. coli	—	±	—	—
B. dysenteriae (Shiga)	—	—	—	—

* *B. amylovorus* had shown slightly inhibited growth in test 4–8–24.

— No inhibitory or lytic effect, medium very turbid.

+ Slight inhibition or lysis, medium turbid.

++ Medium inhibition or lysis, medium slightly turbid.

+++ Strong inhibition or lysis, medium very slightly turbid.

++++ Very strong inhibition or lysis, medium very clear.

Several additional passages and filterings of the material have been made with a rather prompt increase of the potency of the lytic principle. In these tests it has been noteworthy that *Bacterium tumefaciens,* which in the first tests was completely and permanently lysed, has in the later work been only slightly affected. Tubes of this organism to which the lytic principle is added show inhibition of growth for two or three days and then definite pellicle formation occurs, in marked contradistinction to the earlier results. It should be noted that the strain of *Bact. tumefaciens* used is not the same one used in the first experiments.

The additional passages have to date not increased the activity toward any of the animal pathogenes, *Bacillus typhosus* showing consistently only slight inhibition in growth in those tubes to which the lytic principle has been added.

Our experience indicates that while the lytic principle may show activity against a considerable group of organisms, it varies in its effectiveness and seems more active against certain strains than against others. The failure of the lytic principle to lyse the Shiga organism is interesting. The lytic principle seems to lose its potency upon standing.

To test out further the lytic principle obtained from a plant source, cultures of *Bacterium pullorum* Rettger and Harvey and *Bacterium sanguinarium* Moore were obtained through the courtesy of Dr. H. J. Stafseth of the Department of Bacteriology, Michigan Agricultural College. This work was done with filtrates that were very active against *Bacillus carotovorus*. No difference in turbidity of the checks and the cultures to which the lytic principle was added was observed in any of the numerous trials which were made. Invigoration of the material after the methods recommended by d'Herelle (1) were attempted. The two animal pathogenes in some cases were grown in the presence of the lytic principle and in others in the presence of the lytic principle together with the various plant pathogenes against which it was effective. The filtrates from these cultures when tested out against the two animal pathogenes yielded negative results. Similar negative results with these organisms were obtained by Mallmann[3] with his isolations.

RELATIONS TO PLANT PATHOLOGY

The first important rôle which has been suggested by our experiments is concerned with the decline of certain bacterial organisms in the soil. Various workers have noted the decline if not the disappearance of various bacterial organisms in soil, as for example, *Pseudomonas citri* (6). Of special interest is the apparent disappearance of the organism causing potato blackleg (*Bacillus atrosepticus*) from soil in a comparatively short time. The writers have reviewed in another publication the work of Morse, Rosenbaum and Ramsey, and Murphy, as well as given the results of their tests (7). It has been possible to isolate from loam soil which had been drenched heavily with cultures of *Bacillus atrosepticus* a lytic principle which has behaved essentially as the lytic principle isolated from rotted carrot. Similarly a lytic principle has been isolated from river water, which was also effective against *B. atrosepticus* and other plant pathogenes. The writers would conclude that *Bacillus atrosepticus* along with other plant pathogenes, *B. carotovorus* and *B. tumefaciens*, is not refractory toward lysis, that the lytic principles are widely distributed in nature, and that lysis may play an important rôle in decimating or rendering non-virulent these particular organisms. Furthermore, the increase of potency of the lytic principle when exposed to susceptible organisms seems to indicate that the range of organisms concerned in this phenomenon is large. It must be borne in mind that variations in activity with various strains of organisms seem to exist.

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[3] Verbal communication.

The lytic principles can play an exceedingly important rôle in preventing normal infection. Since the early experiments of Jones with the soft rot organism, the rapid and complete rotting of moist carrot disks by *Bacillus carotovorus* has become a common classroom demonstration. Similarly, the rotting of potato slices after inoculation with *Bacillus atrosepticus* follows promptly and with production of a characteristic decay if the tuber slices are kept moist. It is interesting to compare the normal results with those obtained when the lytic principle is introduced as a factor in the experiments.

Fresh, crisp carrots were carefully washed and disinfected with corrosive sublimate. These were sliced into disks with a sterile knife and the disks placed on filter paper in small individual moist chambers freshly steamed, which were in turn held in a larger moist chamber. Five sets of four carrot slices each were used and treated as follows: Series 1. Each slice was inoculated with a loop-drop of sterile nutrient beef broth. Series 2. Each slice was inoculated with a loop-drop of a 48-hour broth culture of *B. carotovorus.* Series 3. Each slice was inoculated with a loop-drop of a mixture made by adding 1 cc. of the 48-hour broth culture used in Series 2 with an equal quantity of the lytic principle obtained originally from carrot. Series 4. This was the same as Series 3, except that the inoculation was made after six hours incubation of the organism with the lytic principle. Series 5. A loop-drop of the lytic principle was spread over the surface of each carrot slice after which they were inoculated with a loop-drop of the 48-hour culture of *B. carotovorus.* In every case the slices were placed in the moist chambers immediately after inoculation. After three days' incubation at room temperature the following results obtained (Table 5 and Plate XI).

TABLE 5—*Protective effect of the lytic principle against Bacillus carotovorus infection: Tests with carrot slices; 3-day period*

Series	Treatment	Results
1	Check, sterile broth	– – – –
2	*B. carotovorus*	+ + + +
3	*B. carotovorus* plus lytic principle, inoculated immediately	– – – –
4	*B. carotovorus* inoculated after 6 hours	– – – –
5	Lytic principle spread on slice just before inoculation	– – – –

– No rotting. + Rotting.

The results from this test were precise and definite. *Bacillus carotovorus* promptly and completely rotted the carrot slices in the check series, whereas none of the slices which had the lytic principle brought in contact with the organism rotted at all. Even with the rather uneven distribution of the lytic material such as would result from spreading a loop-drop over the slice with a platinum needle, rotting was prevented.

In experiments using potato slices and *Bacillus atrosepticus* as the pathogene, similar protective action by the lytic principle was demonstrated. Sound potato tubers were washed and disinfected with corrosive sublimate. These were sliced and placed in individual moist chambers lined with filter paper. A loop-drop of the filtrate containing the lytic principle was spread over the cut surface of each of five slices, two slices being used as checks. Each slice was then inoculated with a loop-drop of *B. atrosepticus* (24-hour culture). The results of this test are shown in table 6a and plate XII.

TABLE 6—*Protective action of lytic principle against B. atrosepticus: Test with potato slices in moist chamber; 3-day period*

a

	Treatment	Result	
Slice 1	ck.	+	Characteristic rot
2	ck.	+	Characteristic rot
3	Plus 1 loop lytic filtrate	–	No rotting
4	'' 1 loop lytic filtrate	+	Characteristic rot
5	'' 1 loop lytic filtrate	–	No rotting
6	'' 1 loop lytic filtrate	–	No rotting
7	'' 1 loop lytic filtrate	–	No rotting
On 3rd. day, Nos. 3, 5, and 7 were reinoculated			No rotting developed

b

Tuber 1,			
Slice 1	ck.	+	Characteristic rot, 15 mm. diam.
2	Plus 1 loop lytic filtrate	+	Characteristic rot; one spot 5 mm. diameter, one 1 × 3 mm.
3	'' 1 loop lytic filtrate	–	No rot
4	'' 1 loop lytic filtrate	–	No rot
Tuber 2,			
Slice 5	'' 1 loop lytic filtrate	–	No rot
6	'' 1 loop lytic filtrate	–	No rot
7	'' 1 loop lytic filtrate	–	No rot
8	ck.	+	Characteristic rot; one area 10 × 20 mm., another 7 × 15 mm.

– No rotting. + Rotting.

The experiment was repeated following the same plan with the results that are shown in table 6b.

The lytic principle when placed upon potato slices operates to prevent the normal process of infection with *B. atrosepticus.* The rotting of a single tuber slice stroked with a loop of lytic filtrate in each of the two tests is not believed to be significant, as by the method employed even distribution of the lytic material can hardly be obtained. When the loop of material is placed upon the slice, the bulk of the drop is deposited at once and spreading must be accomplished by stroking. This militates against reaching all parts of the surface. On the other hand, when the bacteria are placed upon the tuber, the heavy deposit of the bacteria at some one place greatly favors infection at that point.

The writers wish to record their preliminary experiments in using the lytic principle as a protective agency in preventing infections. We believe this work opens up some possibilities in the matter of plant disease control. We believe the relations of the lytic principle to life processes of plant pathogenes needs full and complete investigation. Furthermore, the lytic principle may be a factor playing an important rôle in the infection phenomena in plant diseases of bacterial nature.

SUMMARY

1. The d'Herelle phenomenon has been demonstrated with various plant pathogenes. A transmissible lytic principle (bacteriophage) has been obtained from rotted carrot, from soil, and from river water, which was found to be effective in very high dilutions in causing inhibition of growth of various bacteria and definite lysis in stronger concentrations. The organisms chiefly used were *Bacillus carotovorus, B. atrosepticus,* and *Bacterium tumefaciens.*
2. The potency of these filtrates was increased after a number of passages with a susceptible organism. The lytic principle of eight passages was more effective than the same material of the 4th passage, producing lysis even in dilutions as high as 1:100,000,000.
3. The lytic effects are not static but vary slightly from day to day, especially in tubes with less active lytic principle and with higher dilutions.
4. Loss of motility, malformation, and agglutination are characteristic occurrences in cultures to which the lytic principle has been added.
5. Some evidence that the lytic principle is made up of corpuscles which increase in number upon incubation with a susceptible organism is given. Agglutination seems to be an early evidence of the workings of the lytic principle.
6. Temperature tests, using a range of temperatures from 7.8° C. to 36.1° C., showed that the activity of the lytic principle is greatest at those

temperatures most favorable for the growth of the susceptible organism. The experiments with the plant pathogenes were carried out chiefly at room temperature (25° C.).

7. The lytic principle was polyvalent toward *Bact. tumefaciens, Bacillus carotovorus,* and *B. atrosepticus,* and but slightly active against certain other organisms, such as *B. amylovorus* and *B. typhosus.* It was not active against *Bacillus dysenteriae* Shiga, *Bacterium pullorum,* and *Bact. sanguinarium.* Its activity varied with the strains of the organism tested.

8. The potency of the filtrate was lessened after 5½ months storage in a sealed flask.

9. The occurrence of lytic principles in nature is believed to be widespread, and it is suggested that they play an important rôle in the decline of certain bacterial organisms in the soil.

10. When the lytic principle was spread upon slices from susceptible plants (carrot, potato), infection by *Bacillus carotovorus* and *B. atrosepticus,* respectively, was in whole or in part prevented. This protective action may be of importance in infection phenomena as well as of importance in plant disease control.

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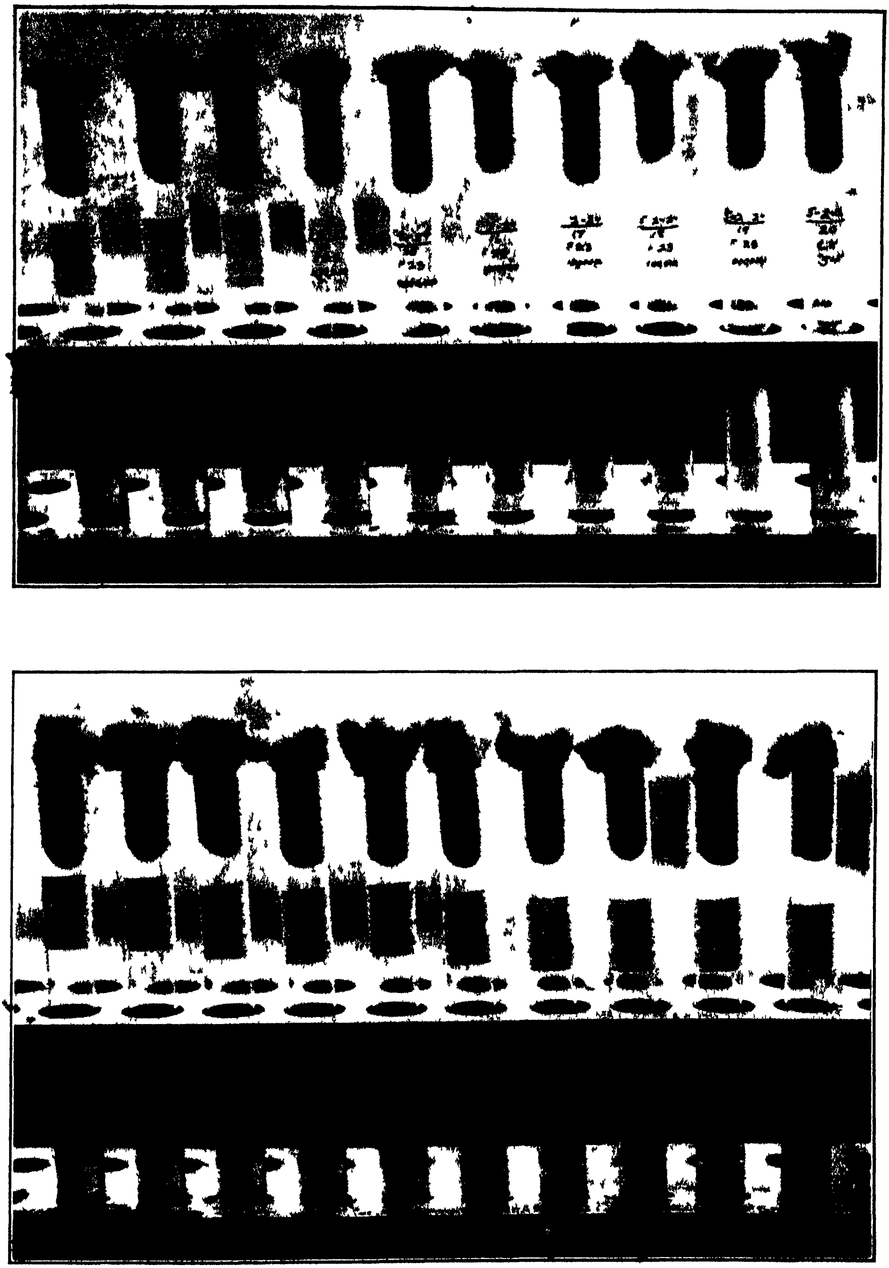

PLATE IX, Figs. 1 and 2. Effect of lytic principle on *Bacillus carotovorus*. (Fourth and eighth serial filtrates compared.)

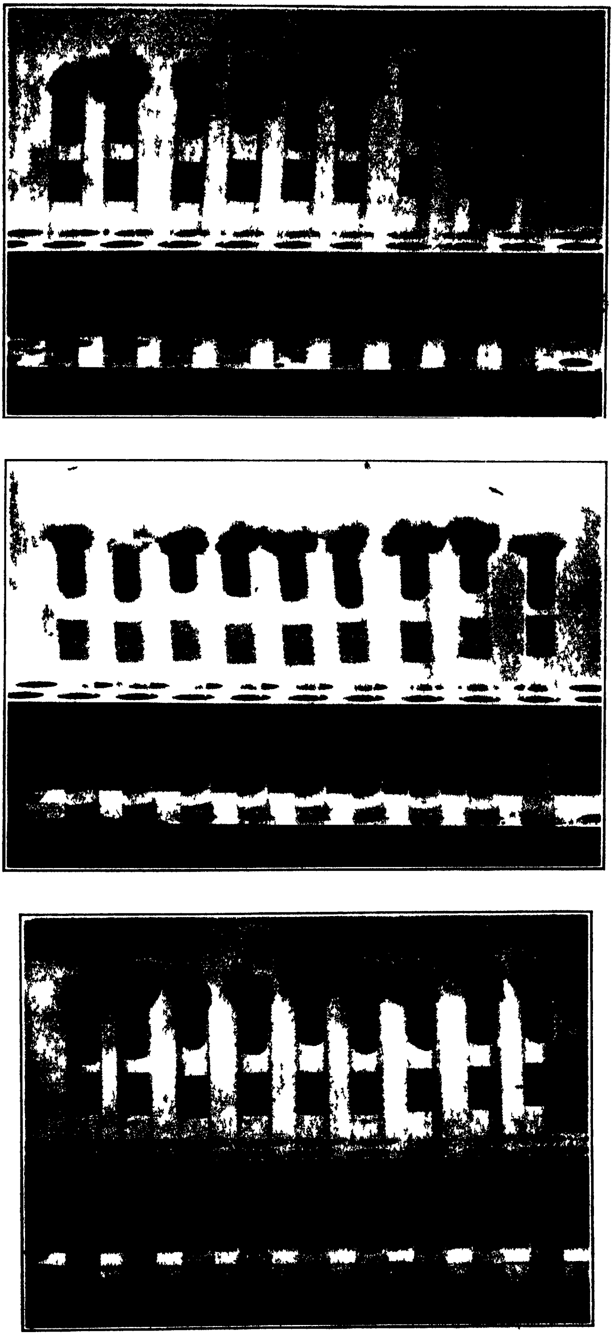

PLATE X, FIGS. 1, 2 and 3. Comparison of the effect of lytic principle on *Bact. tumefaciens*, *Bacillus carotovorus*, and *B. atrosepticus*; 3-day period.

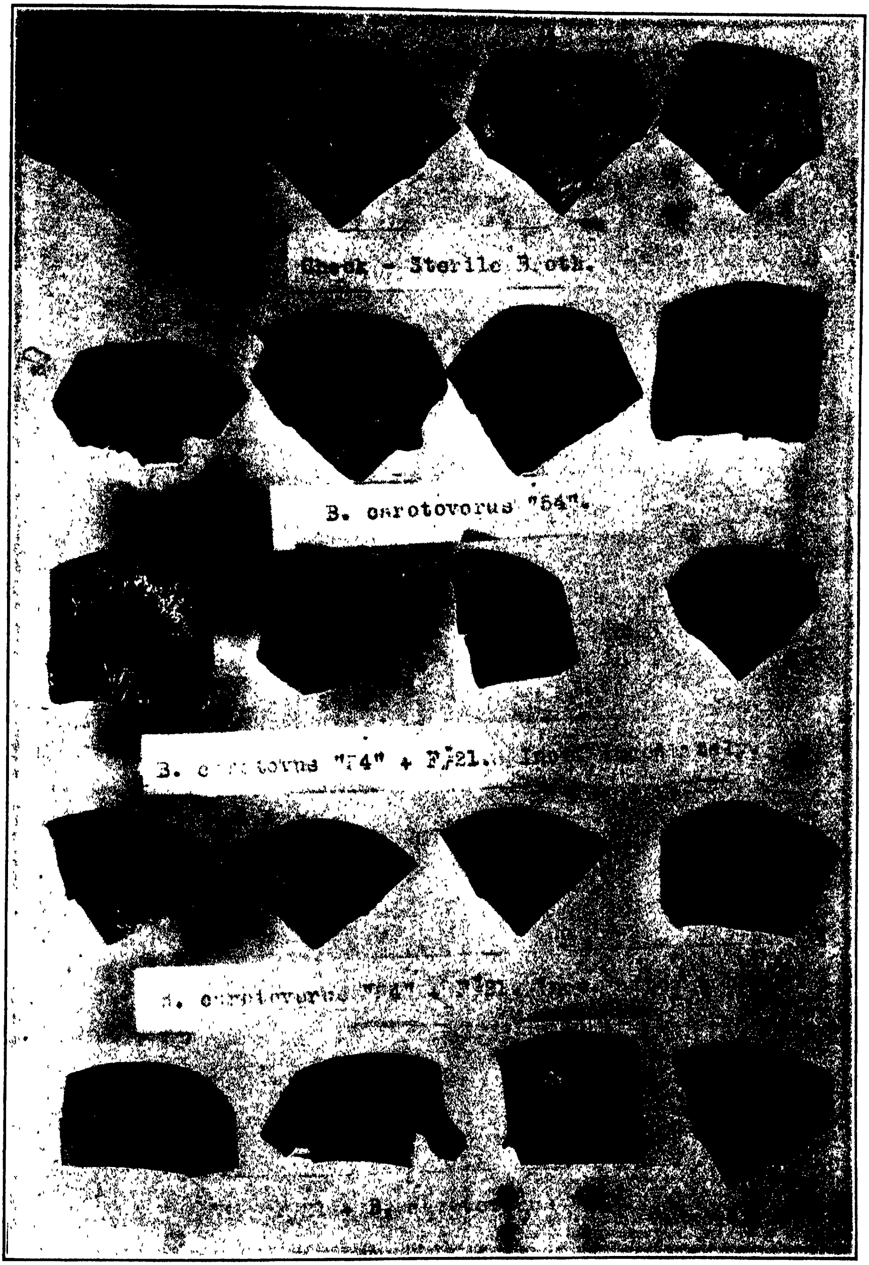

PLATE XI. Protective effect of the lytic principle to raw carrot against infection by *Bacillus carotovorus*.

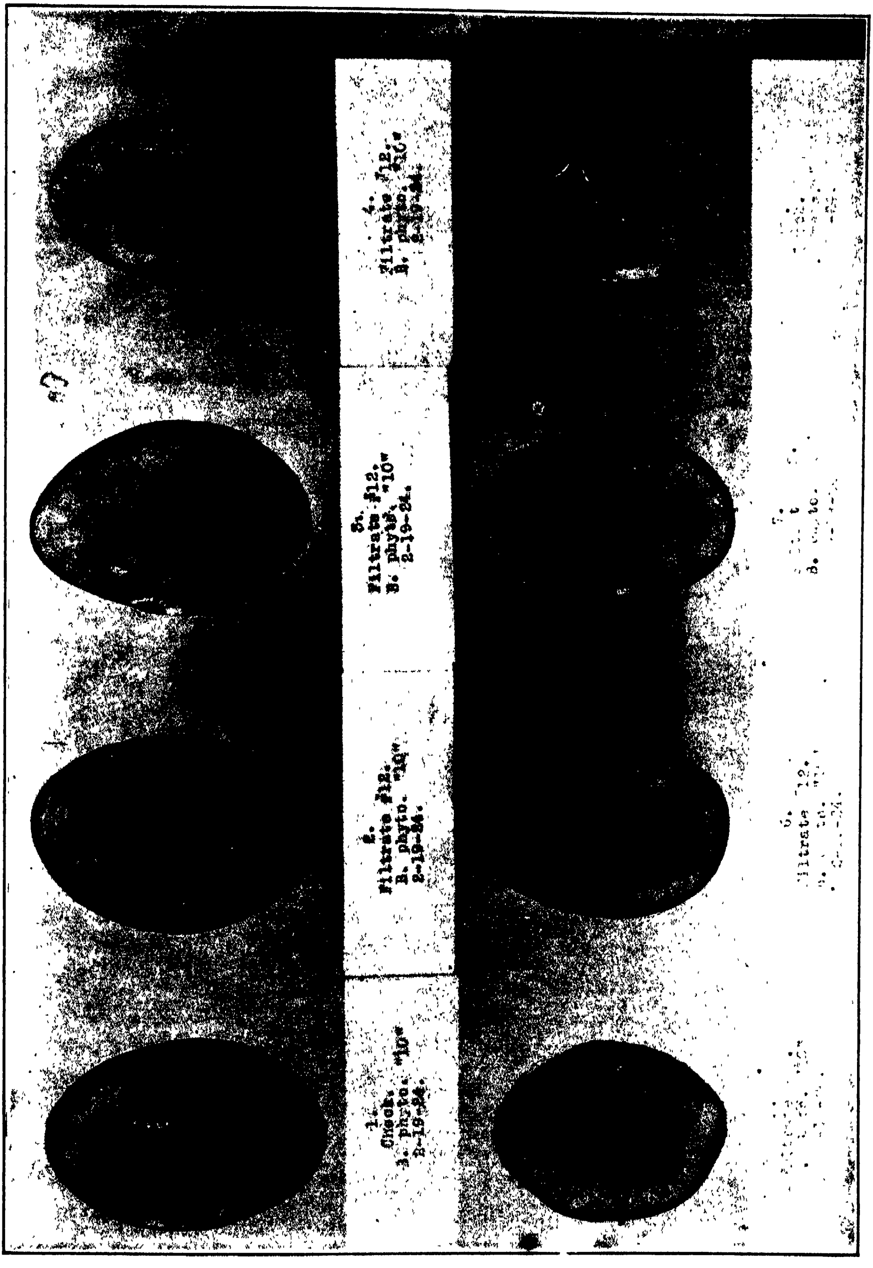
1. Check. B. phyto. #10 2-19-24.
2. Filtrate #12. B. phyto. #10 2-19-24.
3. Filtrate #12. B. phyto. #10 2-19-24.
4. Filtrate #12. B. phyto. #10 2-19-24.

PLATE XII. Protective effect of the lytic principle to raw potato slices against infection by *Bacillus atrosepticus.*

INHERITANCE IN WHEAT OF RESISTANCE TO BLACK STEM RUST[1]

H. K. HAYES, E. C. STAKMAN, AND O. S. AAMODT

WITH PLATE XIII

INTRODUCTION

The problem of developing varieties of wheat which are resistant to stem rust (*Puccinia graminis tritici* Erikss. and Henn.) is complex. Previous studies on the mode of inheritance of resistance and on the pathogenicity of the causal organism have placed the problem on a definite research basis. The present study was made on the inheritance of resistance in a cross between parental material in which there were two types of resistance.

A BRIEF REVIEW OF PREVIOUS STUDIES

It is now known that *Puccinia graminis tritici* consists of forty or more parasitic strains or physiologic forms (10, 11, 15, 16, 17). These physiologic forms can be distinguished by their action in the greenhouse on seedlings of twelve wheat varieties belonging to five groups (17). The following key was devised by Stakman and Levine (17) to indicate the types of reaction of the differential hosts to physiologic forms (Table 1).

Plus and minus signs also are used to indicate fluctuations within a given type. Thus, 3 — indicates that the reaction falls in the 3 class but that it is fairly weak for that class. On the other hand, 3 + indicates that the type of infection is 3 but that it is near the upper limit of the 3 class.

The type of infection on seedlings in the greenhouse is not always the same as that which develops on the same variety in the field. It is difficult to make studies of the reaction of varieties to single rust forms in the field because other forms than the one in question may infect the plants. However, in some seasons when rust was not very prevalent, it was possible to determine the reaction of varieties to particular physiologic forms in the field (5, 6). By correlating the reaction of varieties to several physiologic forms in the greenhouse with the reaction under epidemic conditions in the field, it has been possible to obtain certain information regarding the rela-

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[1] Cooperative investigations between the Sections of Plant Breeding and Plant Pathology of the Minnesota Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

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TABLE 1.—*Explanation of symbols used to indicate types of infection produced by physiologic forms of Puccinia graminis on varieties of wheat*

0—IMMUNE

No uredinia developed; hypersensitive flecks usually present, but sometimes there is apparent absolutely no trace of mycelial invasion in the host tissues.

1—VERY RESISTANT

Uredinia minute and isolated, surrounded by sharp, continuous, hypersensitive, necrotic areas.

2—MODERATELY RESISTANT

Uredinia isolated and small to medium in size; hypersensitive areas present in the form of necrotic halos or circles; pustules often in green but slightly chlorotic islands.

3—MODERATELY SUSCEPTIBLE

Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.

4—VERY SUSCEPTIBLE

Uredinia large, numerous and confluent; true hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable.

X—HETEROGENEOUS

Uredinia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones, and vice versa. Infection ill-defined.

tionship of the reaction of seedlings in the greenhouse to that of older plants in the field.[3]

Stakman and Levine (17) considered that varieties which develop types of infection 0, 1, and 2 in the greenhouses are resistant; while those which develop types 3 and 4 are susceptible. For practical purposes it probably would be preferable to designate plants which develop a 3-type reaction as moderately resistant. It is known definitely that varieties and hybrids on which 0, 1, and 2 types of infection occur in the greenhouse also are resistant to the same forms of rust in the field. But it also is known that seedling plants, apparently susceptible in the greenhouse, are sometimes rather highly resistant in the field. This applies particularly to the 3, 4, and X types of reaction. Studies of the nature of resistance to *Puccinia graminis* may explain these apparent discrepancies.

It is known definitely that there are two types of resistance: (1) a true protoplasmic resistance which varies relatively little, and (2) a morphological resistance which varies with the age of the host and the conditions under which it has grown. Stakman (14) showed that resistance to physiologic forms of *Puccinia graminis* often is due to a real physiological incompatibility between the resistant plants and the invading fungus. He showed that when a germ tube entered a resistant host it apparently secreted some toxic substance which quickly killed some of the cells of the inoculated plant. The fungus itself then died, probably either because of its inability to obtain nutrients from the dead cells or because of the liberation of a harmful substance by the host cells. It was shown that the struggle between host and parasite was short and decisive and involved only a few cells in the most resistant plants. In plants which are only moderately resistant, on the other hand, the rust hyphae do not kill so quickly. Larger areas are involved, which appear as necrotic lesions to the unaided eye, and the rust hyphae are able to live for some time. Uredinia may even be formed, but the development of the rust is distinctly subnormal. In susceptible varieties, however, the fungus apparently does not injure the host cells immediately but actually seems to stimulate them to increased physiological activity. This often results in the production of green islands.

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[3] Harrington, J. B. The inheritance of resistance to *Puccinia graminis tritici* in crosses between varieties of durum wheat. (In press).

Levine, M. N. Statistical studies on the variation of physiologic forms of *Puccinia graminis tritici* and the effects of ecological factors on the susceptibility of wheat varieties. 1924. [Unpublished thesis for degree of Doctor of Philosophy, Univ. Minn.]

Allen (2, 3) confirmed these conclusions and suggested also that many uredinial germ tubes apparently were unable to enter resistant hosts. In other words, certain resistant varieties had two methods of defense against the pathogene: (1) true protoplasmic resistance as described above, and (2) some morphological peculiarity such as size of stomata or some physiological characteristic which made it difficult for germ tubes to enter.

Hursh (8) has proved rather conclusively that wheat varieties may be resistant on account of morphological peculiarities. It is known that the rust mycelium develops almost exclusively in the chlorenchymatous collenchyma of wheat stems. In some varieties the collenchyma bundles are small and are separated by sclerenchymatous fibers. There is therefore a definite mechanical limitation to the spread of the rust mycelium. As the amount of collenchyma is inversely proportional to the amount of sclerenchyma, varieties with a large amount of sclerenchyma would be resistant. There may be no real protoplasmic resistance, but pustules are likely to be small and linear because the areas of host tissue in which the rust can develop are narrow. The amount of sclerenchyma in proportion to collenchyma increases as plants grow older. This, then, would account for the fact that seedlings sometimes may appear to be susceptible in the greenhouse, but older plants of the same variety may be resistant in the field.

Previous studies of crosses in which the two parents react reciprocally to two physiologic forms of rust have demonstrated in all cases that it is possible to obtain homozygous hybrids which are resistant to both physiologic forms, others susceptible to both forms, .s well as others which react in the same manner as the parent varieties (4, 6, 7, 12).

In the present study one parent was immune from certain physiologic forms in the greenhouse, and the other was susceptible in the greenhouse but rather resistant to many forms of rust in the field. The parent varieties were selections from previous crosses. As one of the parents was obtained from a durum-vulgare cross, it appears desirable to discuss the problem of species crosses in relation to rust resistance.

PROBLEM OF SPECIES CROSSES IN WHEAT

Sax (13), Watkins (18), and Kihara (9) have recently carefully studied the problem of wheat species crosses from the cytologic and genetic standpoints. From the standpoint of rust resistance, wheat breeders are chiefly interested in crosses between parent varieties belonging to the emmer and vulgare groups, which contain 28 and 42 chromosomes, respectively. The studies referred to have proved rather conclusively that there is a rapid return to the parental or species chromosome numbers in generations following a cross when varieties belonging to the emmer and vulgare groups are used as parents.

Sterility in generations following the cross has been attributed by Sax to unbalanced numerical relations of the chromosomes, due to random distribution of the univalents and to incompatibility of certain combinations among the bivalents. Of particular interest in relation to rust resistance is the study of Sax in which correlations of chromosome number, morphological characters, and rust resistance were made in the F_3 generation. For the relation between chromosome number and rust resistance, only 37 plants were available. It is very evident from the results presented that the correlation between the 14-chromosome group and rust resistance and between the 21-chromosome group and rust susceptibility was rather close in this study. Of the 12 plants classed as common types and belonging to the 21-chromosome group, one showed considerable resistance, although it was not so resistant as the Kubanka parent. From these studies it is clear that it is difficult to combine the rust resistance of the durum parent with the chromosome number of the vulgare parent when relatively few individuals are available. Sax further concludes that "The characters peculiar to the common wheats are dependent on the assortment of the 7 univalent chromosomes and as a result these characters, in a homozygous condition, are, with few exceptions, found only in segregates with 21 chromosomes." Sax discusses a study made in Minnesota (5) in which over 20,000 F_3 segregates from a cross between rust-resistant durums and common wheats were examined. He emphasizes the important fact that only a few plants were found with the morphological characters of the common wheat parent which were also resistant to rust. In view of the importance of the problem of combining the rust resistance of a durum parent and the desirable characters of the common parent, a brief review of results to date will be of interest.

One of the parents of the cross to be discussed later in this paper is Marquis × Iumillo, N. S. N. II–15–114. This new variety bears the Minnesota Number 2202 and the Cereal Investigations Number 6887. It is the most promising selection obtained from the cross between Iumillo, a red-seeded durum, and Marquis. From unpublished studies made by Dr. Fred Griffee, of the plant breeding section, this hybrid variety is known to have the chromosome number of vulgare wheats. It has been studied rather widely under field conditions and has proved highly rust resistant, although it is not as resistant as some of the better selections of Iumillo. Unfortunately, in the original Iumillo × Marquis cross, the Iumillo parent was not pure, and a reported study (5) of infection of the Iumillo parent showed that the hybrid was as resistant in the greenhouse to physiologic form 1 as the Iumillo parent (C. I. 1736). Numerous selections have been made in Iumillo and some are now available which are much more resistant than the original Iumillo variety.

The difficulties of wheat-species crosses have been emphasized. However, it seems apparent that the possibilities are unknown. The Marquis-Iumillo hybrid II–15–44 is, perhaps, the most promising selection yet obtained from a wheat-species cross. For this reason a summary statement of its performance in relation to Marquis is interesting. Data were obtained on comparative rust reaction in the rust epidemic nursery. The epidemic was induced by the use of all available physiologic forms of *P. graminis tritici.* The Marquis × Iumillo hybrid appears rather highly resistant (see table 2).

TABLE 2.—*Rust reaction of Marquis, Iumillo, and Marquis × Iumillo II–15–44*

Year	Physiologic forms available	Percentage rust infection		
		Marquis	Marquis × Iumillo	Iumillo
1920	1, 3, 9	90	5	0
1921	1, 3, 9, 14, 17, 18, 19, 21, 29, 32	50	2	0
1923	1, 3, 9, 11, 17, 18, 19, 21, 34	70 S	15 R & SR	15 R
1924	1, 3, 9, 17, 18, 19, 21	50 S	Tr	0

The method of indicating rust reaction in percentage of infection has been described previously. In recent years, in the rust nursery, the letters, S, SR, and R also have been added. These refer to the type of infection. Very small uredinia, as well as long linear ones are considered to indicate resistance (R). An intermediate type which appears less well developed than the susceptible group is designated as semi-resistant (SR) while the presence of normal, large, healthy uredinia indicates complete susceptibility (S).

Data on yielding ability and rust reaction are given in table 3. Appearently the hybrid variety is rather highly resistant under field conditions in many localities. At University Farm, in 1924, there was a very serious infection with root-rot. The durum varieties are particularly susceptible. While not so severely injured as durum wheats the new hybrid, II–15–44, was more severely injured than Marquis.

The hybrid was rather highly resistant in the various field studies, and in years when rust is destructive it yields much better than Marquis.

Preliminary milling and baking trials of the hybrid and of Marquis have been made under the direction of Dr. C. H. Bailey, of the Department of Agricultural Biochemistry, University of Minnesota. The seed for the milling trials was obtained from rod-row plots. In 1922 seed was available from University Farm only, while in 1923 comparative milling tests were made of University Farm material and also of a mixture of equal portions of seed obtained from the Morris, Crookston, and Waseca branch stations. The seed of Marquis grown at University Farm was fairly plump while that from the branch stations was severely injured by black stem rust.

TABLE 4.—*Milling and baking results of Marquis and Marquis × Iumillo II–15–44*

Variety or Hybrid	Year grown	Place grown	Flour						Plumpness of grain
			Protein	Loaf volume	Water used	Texture score	Color score	Total flour	
			pct.	cc.	cc.			pct.	pct.
Marquis × Iumillo 11–15–44	1922	U. Farm	13.05	1540	58.1	98	99	75.8	83
Do	1923	U. Farm	16.73	2110	56.4	97	96	67.8	84
Do	1923	C. M. W.	16.25	1940	57.7	96	95	73.6	79
Average			15.34	1863	57.4	97.0	96.7	72.4	82
Marquis	1922	U. Farm	12.03	1590	59.9	99	99	74.0	80
Do	1923	U. Farm	16.16	2000	57.8	99.5	99.5	68.6	79
Do	1923	C. M. W.	15.65	1860	57.3	94	97	70.7	39
Average			14.61	1817	58.3	97.5	98.5	71.1	66

A comparison of the baking tests indicates that the hybrid is nearly the equal of Marquis in baking quality. Probably the hybrid is slightly inferior to Marquis in its color score. The results justify the conclusion that it is possible to combine in a common wheat the rust resistance obtained from a durum wheat parent and the milling quality of the common wheat parent. It is true that the hybrid appears slightly inferior to Marquis but the differences are very small.

Another series of crosses was made in 1920 between Mindum, a variety of *Triticum durum*, and Marquis and Velvet Chaff, two varieties of *T. vul-*

TABLE 3.—*Comparative yielding ability and percentage of rust infection of Marquis and Marquis × Iumillo II-15-44**

Type of plot	Locality	Year of test	Yield, bushels per acre		Percentage rust infection	
			Marquis	Marquis × Iumillo	Marquis	Marquis × Iumillo
Rod rows	Univ. Farm, St. Paul, Minn.	1921	18.4	16.1	0	0
"	" " " " "	1922	36.0	40.0	41	T
"	" " " " "	1923	31.0	32.4	22	T
"	" " " " "	1924	26.9	22.7	0	0
"	Waseca, Minn.	1922	19.7	29.1	35	3
"	" "	1923	22.2	22.6	23	4
"	Crookston, Minn.	1922	28.1	26.8	20	2
"	" "	1923	15.8	21.8	58	T
"	Morris, "	1922	24.8	27.2	24	0
"	" "	1923	16.4	21.8	88	15
"	Ave. of 6 localities, Canada	1923	28.2	37.4	30–75	5–15
"	" " " " "	1924	28.8	26.2	0–45	0–3
"	Mandan, N. D.	1924	21.8	22.6	24	3
"	Dickinson, N. D.	1923	11.2	15.7	50	20
"	" " "	1924	20.9	11.8†	25	5
"	Moccasin, Mont.	1923	36.6	37.7	0	0
"	" "	1924	36.9	32.4	0	0
"	Fargo, N. D.	1924	38.4	37.0	64	2
"	North Platte, Neb.	1924	31.4	25.1	0	0
1/40 Acre	Crookston, Minn.	1924	20.5	30.0	95	50
"	Morris "	1924	28.9	27.9	42	1
"	Waseca "	1924	37.2	42.1	80	1
"	Grand Rapids, Minn.	1924	15.0	15.4	87	42
"	Univ. Farm, St. Paul, Minn.	1924	33.3	32.0	0	0
"	Moccasin, Mont.	1924	32.0	31.5	0	0
"	Brandon, Man.	1924	44.0	55.3	70	20

* Acknowledgement is made to various investigators who have kindly furnished comparative data which they have taken. Through such cooperation a comparison under many different conditions has been made possible. The data obtained from 6 places in Canada were furnished by Prof. W. P. Fraser; Mr. J. A. Clark, of the U. S. Dept. of Agriculture, furnished data from Mandan and Dickinson, North Dakota, and Moccasin, Montana; Professor L. R. Waldron furnished the data from Fargo; Mr. S. J. Sigfusson furnished the report from Brandon, Manitoba, Canada.

The data collected at the branch stations in Minnesota were obtained from Prof. A. C. Arny for the 40th-acre plot tests. The various branch station agronomists, R. E. Hodgson, Waseca; R. O. Bridgford, Morris; R. S. Dunham, Crookston; O. I. Bergh, Grand Rapids; and M. J. Thompson, Duluth, assisted in the collecting of data obtained from the branch stations.

† The low yield was probably a result of root-rot.

gare. It was hoped to make a careful study of fruitfulness of the F$_2$ hybrids in relation to plant type, and, in order to prevent cross pollination, spikes of each plant were covered with a glassine bag. The season of 1921 was not very favorable and for this reason the results have not been studied in detail. It also was learned that the degree of fruitfulness of Mindum was much more seriously affected by covering with a paper bag than that of Marquis and this interfered with a careful genetic analysis of the data. As in the previous study, the various plants were placed in seven general groups, largely on the basis of keel development, *viz*: durum, near-durum, intermediate, near-common, common, emmer-like, and emmer. The plants of the two groups, common and near-common, were selected and their progeny grown in F$_3$. Forty-four lines were available. Fourteen lines which appeared promising, and which appeared to be breeding true for the vulgare habit, were harvested, several plants from each line being chosen. These were threshed individually and the reaction of their F$_4$ progeny was tested in the greenhouse to two physiologic forms of rust to which Mindum was resistant and Marquis susceptible. The results of this trial are presented in table 5.

TABLE 5.—*Reaction of Marquis, Mindum, and fourteen F$_4$ lines of Marquis × Mindum or Marquis × Velvet Chaff to two physiologic forms to which Marquis is susceptible and Mindum resistant*

Parents or crosses	Types of reaction to physiologic forms	
	1	18
Mindum Marquis	Flecks and 1 4	Flecks and 1 4
Marquis or Velvet Chaff × Mindum, F$_4$, 12 families	All produced some 4's. A few families were heterozygous, the remainder produced only susceptible plants.	All susceptible
Velvet Chaff × Mindum, F$_4$, No. 70	Flecks, 1, and a few 3	Mostly 4
Marquis × Mindum, F$_4$, No. 25	1 and 2	1

All investigators who have studied species crosses agree that vulgare types, as a rule, can be determined by inspection, although further studies of spike morphology and cytological condition should be made. The seedlings of one family of vulgare appearance were resistant to both physiologic forms 1 and 18 in the greenhouse. Another family was susceptible to form 18 and resistant to form 1. The Velvet Chaff parent was not tested. The remaining 12 families were homozygous for susceptibility to form 18 and either heterozygous or susceptible in their reaction to form 1. These data furnish further proof of linkage between the vulgare characters and susceptibility in crosses between resistant durums and susceptible commons. They indicate that the linkage is not absolute and furnish a further reason for the hope that rust-resistant common wheat can be obtained from crosses between susceptible varieties of *T. vulgare* and resistant varieties of *T. durum.*

Besides presenting data in relation to the possibilities from wheat species crosses, the purpose of the present paper is to describe the mode of inheritance of rust resistance in a cross in which the parents were known to have two different types of rust resistance. It is known that the resistance of the Marquis-Kanred hybrid is protoplasmic. Whether the resistance of the Marquis-Iumillo parent is physiologic or morphologic in nature, or both, is not known definitely.

GENETIC FACTORS INVOLVED IN TWO TYPES OF RUST RESISTANCE

The study of inheritance of resistance was made in the F_2 to F_4 generations of a double cross (Marquis × Iumillo) × (Marquis × Kanred). The Marquis × Iumillo parent is the strain already described under the N. S. N. II–15–44 which, under field conditions, has proved rather resistant to various physiologic forms of *P. graminis tritici.* It obtained this resistance from the durum variety, Iumillo. Two different Marquis × Kanred hybrid lines were used as parents. Both reacted under greenhouse conditions in the same manner as their Kanred parent. From previous studies they are considered to be immune from eleven of the twenty-one physiologic forms found in the north-central spring wheat section (1). The purpose of the study was to learn the factors of resistance involved and the possibilities of combining in one variety the resistance of both parents. The problem was one phase of an attempt to produce desirable spring wheat varieties resistant to black stem rust. Although the Marquis × Iumillo parent has been rather resistant under field conditions in all tests so far made, it shows little or no resistance in the seedling stage to various physiologic forms of rust when the study is made under greenhouse conditions. Seedlings of the two Marquis × Kanred parents are both immune, in the greenhouse, from the physiologic forms from which Kanred is immune. As both of the Kanred × Marquis strains used as parents reacted in a similar manner, and as their progeny segregated in a similar manner in the crosses with Marquis × Iumillo, the results have been combined in the tables.

The study of inheritance was carried on with approximately 250 F_3 lines from the double cross (Marquis × Iumillo) × (Marquis × Kanred). These F_3 lines represented a random sample of F_2 plants not previously studied for their rust reaction. It is obvious that the most accurate means of determining the genotypic condition of F_2 plants with respect to disease resistance or susceptibility is to study the breeding behavior of F_3 lines.

In previous studies it was learned that the immunity of Kanred was a dominant character and that immunity from all physiologic forms from which Kanred was immune was dependent on a single genetic factor. The study of inheritance of the Kanred type of immunity in the double cross (Kanred × Marquis) × (Marquis × Iumillo) was made by growing from 20 to 40 seedlings of each F_3 hybrid line in the greenhouse and inoculating them with spores of form 21. As in previous studies, a single genetic factor pair differentiated immunity and susceptibility and the F_3 lines were placed in three groups: I, homozygous for immunity, producing types of infection only in the 0 class; H, heterozygous, producing both 0 and 4 types of infection with a ratio of approximately 3 immune to 1 susceptible; and S, producing only susceptible types of infection in the 3 and 4 classes.

The same hybrid lines were grown under field conditions and an artificial epidemic was induced by inoculating with forms 1, 3, 9, 17, 18, 19, 21, and 34. Physiologic form 11 was obtained from a culture taken from the rust nursery and therefore was present as a result of natural infection. The heavy epidemic in 1923 made it possible to differentiate clearly between resistance and susceptibility. However, there often is no sharp line between resistance and susceptibility under field conditions. The various families from which approximately 25 seeds were sown were studied by the individual-plant method, the plants being placed in three groups according to type and extent of infection as follows:

R, Resistant, with only a relatively small amount of injury from rust. The uredinia produced are small or of the narrow-linear type.

SR, Semi-resistant, with uredinia of an intermediate type of development.

S, Susceptible, with normal, well-developed uredinia.

Rows of Marquis wheat were grown throughout the series and were similarly studied. This variety was completely susceptible throughout.

The Kanred-Marquis parent was somewhat resistant under field conditions. This resistance was not due to the immunity from certain physiologic forms but to a tendency to resistance characteristic of some Kanred hybrids. The resistance, however, can not be studied accurately as it is not striking enough to be differentiated from fluctuations due to environmental conditions. Under field conditions the hybrids and parent lines were placed in groups according to their reactions. In most cases enough plants were available to give an accurate indication of the genotypic condition. Susceptibility proved to be dominant and therefore the classification of resistant lines was probably approximately correct.

Some idea of the accuracy of the investigation can be gained by a study of the behavior of the parent cultures which were distributed throughout the greenhouse and field and which were studied in the same manner as the hybrids. Under greenhouse conditions, the parents and hybrids were classed as homozygous for immunity (I); heterozygous, with immunity dominant (H); and susceptible or homozygous for susceptibility (S).

The F_3 lines grown in the field were studied by the individual plant method. The Marquis × Iumillo, II–15–44, parent was resistant, although approximately half of the plants were more severely infected than the others. None, however, was as susceptible as Marquis. About half of the plants in each row of the Marquis × Iumillo parent were classed as SR and the remainder as R. This was considered as the expected phenotypic expression of the factors for resistance of the Marquis × Iumillo parent. Classes for field reaction were as follows:

R, Resistant or with approximately half of the plants R and the remainder SR, and with no S plants.
H, Heterozygous, with a small percentage of plants in the R group, the greater proportion being classed as SR or S.
In, Intermediate, or all plants with the SR type of infection.
S^1, Containing both SR and S types of infection.
S, Susceptible, containing only S types of infection.

The classification of the reaction of the parent lines under greenhouse and field conditions is given in table 6.

TABLE 6.—*Classification of parent lines, Marquis × Iumillo and Marquis × Kanred, on the basis of their reaction to stem rust under greenhouse and field conditions*

Parent	No. of separate tests	Reaction to physiologic form 21, in the greenhouse	Field reaction
Marquis × Iumillo	8	S	R
" "	1	S	H
" "	2	H	R
" "	2	I	In
Total	13		
Marquis × Kanred	5	I	S^1
" "	6	I	In
" "	1	I	H
" "	1	F	S^1
" "	1	H	In
Total	14		

The four cultures of Marquis × Iumillo, classed as H or I on the basis of the type of infection in the greenhouse, probably escaped infection. The 0 type of infection predominated on the two lines of the Marquis × Kanred parent which were classed as H. Of the Marquis × Iumillo parent lines under field conditions, 10 out of 13 were classed as resistant. This shows the degree of accuracy with which it was possible to classify the various lines under the conditions of the experiment. Experience in studies where physiologic forms are used leads to the belief that the results may be accepted in general as accurate indications of the nature of the genetic factors involved. In order to be absolutely sure of a particular classification from the breeding standpoint, a particular line must be studied through several generations.

The various hybrid families are classed according to greenhouse and field types of infection by the method already described. The results of this study are given in table 7.

TABLE 7.—*Classification of 249 F_3 families of the cross of (Marquis × Iumillo) × (Marquis × Kanred) on the basis of the reaction of seedlings to physiologic form 21 in the greenhouse and of older plants under field conditions to an epidemic of stem rust produced artificially by several physiologic forms*

	Number of F_3	Classification for greenhouse reaction	Classification for field reaction
	6 + 1*	I	R
	19	I	H
	8 + 1	I	In
	15 + 2	I	S^1
	3	I	S
Total	51 + 4		
	10 + 1	H	R
	45 + 2	H	H
	15 + 1	H	In
	55 + 4	H	S^1
	6	H	S
Total	131 + 8		
	0 + 2	S	R
	7	S	H
	3	S	In
	32 + 2	S	S^1
	9	S	S
Total	51 + 4		

* The families classed as + 1, etc., are of doubtful nature chiefly because of small numbers involved.

It is apparent that only a single factor pair is involved for reaction to physiologic form 21 under greenhouse conditions. Under field conditions, 16 families with a +4 reaction in the greenhouse out of a total of 249 are classed as resistant, with the type of resistance of the Marquis × Iumillo parent. This ratio agrees fairly well with a 15 : 1 ratio and indicates that the Marquis × Iumillo type of field resistance is dependent on the interaction of two genetic factors, both of which must be present in a homozygous condition to produce resistance. The allelomorphs of these factors, either alone or in combination and apparently in either the homozygous or heterozygous condition, lead to susceptibility.

Besides the F_3 generation lines which were grown in 1923, F_2 families in which Marquis × Iumillo was one parent have been studied under rust conditions. Most of the plants have been susceptible, although some resistant plants appear in such F_2 progenies. These facts furnish further proof that more than a single genetic factor determines resistance of the Marquis × Iumillo parent in the field.

The genetic factors for resistence of the Marquis × Iumillo parent under field conditions are inherited apparently independently from the factor for immunity from certain physiologic forms under greenhouse conditions, although the nature of the material is such that a loose linkage could not be demonstrated. At any rate, it is apparent that any combination of the parental types of resistance or susceptibility can easily be obtained in the progeny.

One F_3 family appeared more resistant than others. Desirable plants from resistant families and resistant plants obtained from susceptible families were selected and their F_4 progeny were grown in 1924. With the exception of one or two lines, the 1924 progeny appeared rather highly resistant, although some lines appeared more resistant than others. The resistance appeared much greater than in previous tests of F_4 Kota × Marquis crosses, indicating that perhaps fewer genetic factors were involved in the resistance of Marquis × Iumillo than for Kota wheat. One F_3 line, the most resistant of all, was tested in F_4 and again proved more resistant than the other lines.

While there is an indication of two main genetic factors, modifying factors also apparently are involved.

DISCUSSION OF RESULTS

The discovery of physiologic forms of stem rust aided materially in placing the breeding of rust-resistant varieties on a definite genetic basis. The problem is obviously one of combining in a single variety of wheat the resistance of particular wheat varieties by appropriate crosses and recrosses.

While some common wheats of the *T. vulgare* group are resistant to certain physiologic forms under field conditions, it is apparent that varieties of *T. dicoccum* and *T. durum*, of the emmer group, are most resistant. The Marquis × Iumillo hybrid used as one parent in the present study obtained its resistance from the durum parent, Iumillo. From crosses between Mindum, a durum variety, and Marquis and Velvet Chaff, 14 F_3 families were obtained which appeared to be homozygous common types. This classification was made on the basis of keel and spike characters, although the keel was the main means of classification. F_4 progeny of these families were tested in the greenhouse to two physiologic forms of rust to which Mindum was resistant and Marquis susceptible. One family, which was tested by studying the progeny of three plants, was resistant to both physiologic forms of rust. This family was the result of a cross between Marquis and Mindum. One family of Velvet Chaff × Mindum was resistant to one physiologic form and susceptible to the other, while the remaining families were predominantly susceptible, although occasional plants were less severely infected than others.

These results indicate that it is possible to transfer rust resistance from the 14-chromosome group to the 21-chromosome group. That no commercial varieties have yet been obtained from such crosses perhaps may be due to the large number of factors involved as well as to chromosome behavior. The genetic difficulties are so great that one can hardly expect to obtain a segregate which contains all desired characters, especially when the parents differ in chromosome number and many characters of economic importance. It has been demonstrated by these studies, however, that rust resistance can be transferred from the 14-chromosome group to the 21-chromosome group.

In the crosses of Marquis × Iumillo with Marquis × Kanred there apparently were two factors involved for the Marquis × Iumillo type of resistance. On the chromosome basis of heredity it seems logical to conclude that at least two chromosomes or parts of chromosomes which contained factors for rust resistance were obtained from the Iumillo durum parent and that these were combined with chromosomes of the common group, which led to the production of a rust-resistant common wheat with 21 chromosome pairs.

Because of the obvious importance of the problem it is hoped that more intensive studies of the genetic and cytologic phases of wheat-species crosses may be made in the future. In the meantime the results reported give further hope that it may be possible to combine in a 21-chromosome wheat the important characters of bread wheats together with certain desirable durum characters such as rust resistance.

SUMMARY

1. In order to obtain wheat varieties resistant to all physiologic forms of stem rust it is necessary to make crosses and recrosses. The purpose of such crosses is to obtain a desirable variety of *T. vulgare* resistant to all physiologic forms prevalent in the area growing spring wheats.

2. Certain durum and emmer wheats are more generally resistant to the physiologic forms than any known varieties of the common group. The Marquis × Iumillo hybrid, II–15–44, was obtained from a cross of Iumillo durum and Marquis. So far as tested, it is rather rust resistant under field conditions, yields well, and is not apparently greatly inferior to Marquis in milling quality, although more milling and baking tests are needed before final conclusions should be drawn.

3. The importance of a knowledge of the possibilities of combining in a variety of *T. vulgare* the desirable qualities of bread wheats, together with such characters as rust and drouth resistance which can be obtained from durum varieties, emphasizes the necessity of further intensive and extensive genetic and cytologic studies of wheat-species crosses.

In a cross of the common wheats, Marquis and Velvet Chaff with Mindum, a durum wheat, fourteen F_3 families of apparent vulgare habit were selected. The reaction of the F_4 progeny of these 14 families to two physiologic forms to which Mindum was resistant and Marquis was susceptible was determined under greenhouse conditions. One family of the Marquis × Mindum cross, resistant to both physiologic forms, was obtained. This resistance was derived from its durum parent. These facts, together with the Marquis × Iumillo hybrids, prove the possibility of obtaining rust resistance from a durum parent and combining it with the chromosome number of common wheats.

4. In a double cross, (Marquis × Iumillo) × (Marquis × Kanred), the inheritance of two types of resistance was studied. The Marquis × Iumillo parent is resistant to many physiologic forms of stem rust under field conditions, but it is susceptible in the seedling stage in the greenhouse. The Marquis × Kanred parent is immune from 11 of the 21 physiologic forms found in the hard red spring wheat region both in the greenhouse and under field conditions. The Marquis × Kanred type of immunity is dependent upon a single factor pair, while at least two factors are necessary to explain the resistance of the Marquis × Iumillo parent. The factors for resistance of the Marquis × Iumillo parent apparently are inherited independently of the factor for immunity of the Marquis × Kanred parent.

5. All combinations of resistance and susceptibility of the parents were obtained in the hybrids. Homozygous types were obtained which con-

tained the factors for resistance from both parents, as well as homozygous types which were susceptible both under greenhouse and field conditions.

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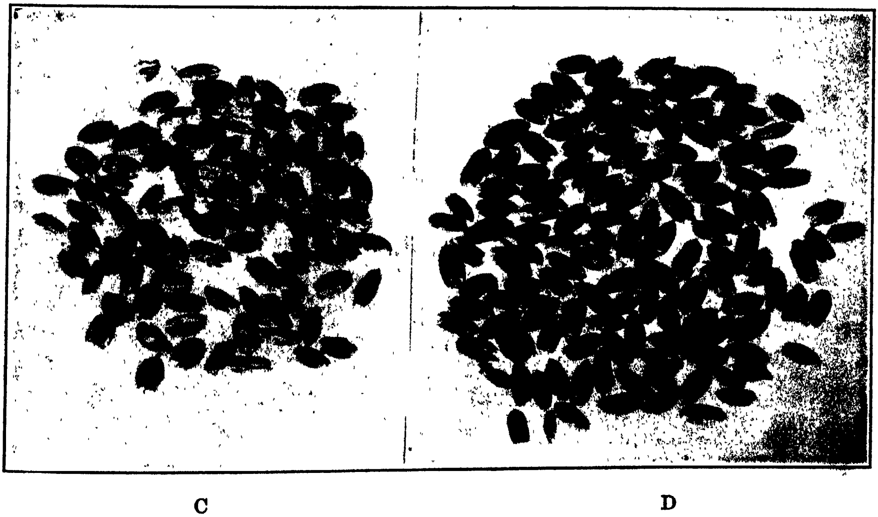
EXPLANATION OF PLATE XIII

Comparison of Marquis and an F_3 hybrid family of the double cross of (Marquis × Kanred) × (Marquis × Iumillo) under artificially induced epidemic conditions in the rust nursery.

A.—Marquis check; B.—An F_3 family which was also highly resistant in F_4; C.—Seed of Marquis check row; D.—Seed of the F_3 hybrid.

PLATE XIII



FURTHER STUDIES ON PYTHIACEOUS INFECTION OF DECIDUOUS FRUIT TREES IN CALIFORNIA

RALPH E. SMITH AND ELIZABETH H. SMITH

WITH SIX FIGURES IN THE TEXT

A phycomycetous fungus causing a serious rotting of lemons in California was described by the writers (7, 8) in 1906 as a new genus and species, *Pythiacystis citrophthora*. *Pythiacystis* was distinguished from *Pythium* by the formation of swarm spores within the sporangium and the absence of a vesicle, and from *Phytophthora* mainly by the fact that it seemed normally to be a moist soil-inhabiting saphrophyte, rather than an aerial parasite. In the affected tissue of the lemon only sterile mycelium of the fungus was found. Infection originated from zoospores produced in the soil beneath the tree. The statement was made that *Pythiacystis* "forms a close transition from Pythium to Phytophthora" and that it is "more exactly intermediate between the Saprolegnieae and Peronosporeae than Pythium." No oospores or chlamydospores were found. The difference in habitat and parasitic relations between this and typical *Phytophthora,* such as *P. infestans,* seemed sufficient grounds in 1906 for distinguishing *Pythiacystis* from that genus. *Pythiacystis* seemed as close to the Pythiaceae and the Saprolegniales as to *Phytophthora* and the Peronosporales.

The only species of *Phytophthora* in which a soil relation of any significance had been suggested previous to 1906 were *P. omnivora* de Bary (*P. cactorum* (Leb. and Cohn.) Schroet., *Peronospora sempervivi* Schenk, and *Phytophthora fagi* R. Hartig) (1, 2, 3), and *P. nicotianae* Van Breda de Haan (4). In these cases it had been shown that the fungus in the soil may be the source of infection of living plants; but that saprophytic soil or semi-aquatic life is the usual habitat, and that normal sporulation is limited to such conditions were by no means indicated.

Since 1906 a number of new species of *Phytophthora* and new diseases caused by old ones have been described and several have been met with by the writers, the nature of which points strongly toward close relationship between *Pythiacystis* and *Phytophthora,* as the latter genus is now understood, and throws great doubt on the desirability of continuing to regard the former as a distinct genus. Several writers have already suggested such a change (23, 29, 49), but without formally proposing it. Some of these new species also form a close connection between *Pythium* and *Phytophthora* and support the opinion expressed by Fitzpatrick (49) that

Pythium, Phytophthora, and *Pythiacystis* should be merged into one genus distinct from the Peronosporaceae (*Peronospora, Plasmopara,* etc.) in their absence of well-defined conidiophores and their saprophytic tendencies. Some of the principal species and diseases of this nature which have been described since 1906 are as follows:

Phytophthora cactorum (Leb. and Cohn) Schroet. (*P. omnivora*) (6, 10, 12, 14, 15, 24, 28, 29, 36, 38, 40, 45, 54); *P. nicotianae* Van Breda de Haan (36); *P. syringae* Kleb. (5, 9, 20, 46, 47, 53); *P. faberi* Maub. (11, 33, 52, 54, 56); *P. arecae* (Colem.) Pethy. (13, 20); *P. parasitica* Dastur (16, 50); *P. erythroseptica* Pethy. (19, 34); *P. allii* Sawada (25, 32); *P. melongenae* Sawarda (25, 32); *P. terrestris* Shreb. (31, 35, 39, 41, 43, 48); *P. cryptogea* Pethy. and Laff. (37, 39), and *P. mexicana* Hotson and Hartge (51).

In these cases such effects as rotting of fruit, damping off of seedlings, root rot, stem and twig blight, and similar effects were pronounced. All of the fungi mentioned have been shown to be capable of saprophytic soil life and able to cause infection by contact of plant tissues with the earth or by zoospores produced therein.

In addition to the definite species listed, numerous instances are mentioned in the literature of strains or forms of fungi having similar habits and effects, but differing in morphological and other characteristics from the typical species. In fact, the discovery of such a variety of strains has been the rule rather than the exception in the investigation of many of these Phytophthora diseases. Several different strains or species are commonly found causing apparently the same disease on the same host, and the identification, delimitation, and validity of species are in many cases still considerably in doubt. Oospores have been found in all the species listed except *P. faberi,* in which case chlamydospores are abundant.

LATER KNOWLEDGE OF PYTHIACYSTIS CITROPHTHORA

Fawcett (17, 18, 23, 41) showed that this fungus is the cause of a very prevalent type of trunk gummosis of lemon trees in California. Fungi causing foot rot (Mal di Gomma) of orange trees (23, 31, 35, 41) and stem canker of avocado (27, 29, 41) were identified as *P. citrophthora,* but later found to be more typical of *Phytophthora,* having oospores and characters resembling those of *P. terrestris* or *P. cactorum.* Barrett (29) reported the isolation of three strains of a fungus very similar to *Pythiacystis citrophthora,* but with oogonia, oospores, and antheridia similar to those of *Phytophthora cactorum.* True *Pythiacystis citrophthora* isolated from California lemons has been grown in culture by a number of investigators in addition to the writers and Fawcett, including Sawada (25), Barrett (29), and Sherbakoff (31), and found to be distinct from any form of *Phytophthora* studied by them. No sexual bodies and no chlamydospores have ever been found in what may be called typical *P. citrophthora,* but the series of Pythiacystis-Phytophthora-connecting strains discussed in this paper represents a variation which seems very typical of that found by many other investigators.

In 1915 a serious infection of the bark of pear, peach, and almond nursery trees was reported by one of us (26), in which typical *Pythiacystis citrophthora* was found. The fungus produced no spores of any kind in the host tissue and no oospores or chlamydospores in culture. This will be referred to as strain A. There was also obtained from almond trees attacked by the same disease a fungus resembling *Pythiacystis,* but with a somewhat different growth in cultures and production of oospores on agar. This will be designated as strain B.

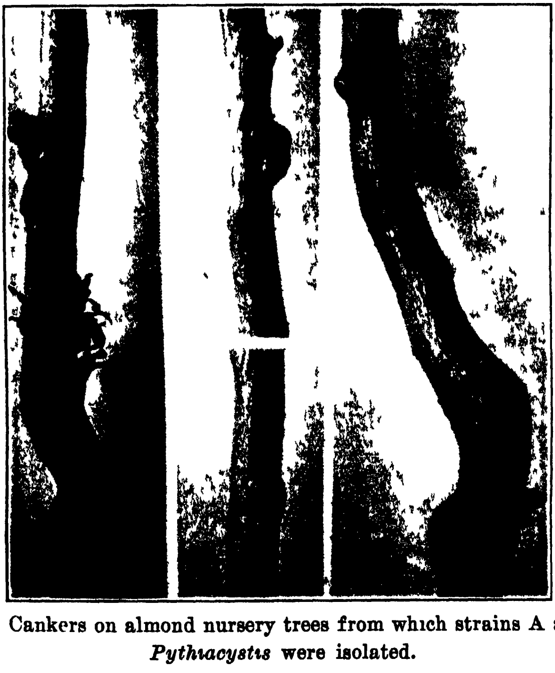


FIG. 1. Cankers on almond nursery trees from which strains A and B of *Pythiacystis* were isolated.

INOCULATION EXPERIMENTS WITH NURSERY TREES

In the initial experiments, strain B failed to develop when inoculated into pear trees. During the winter of 1916–17 further inoculations were made on nursery trees to test the comparative virulence of the two strains. In these trials, cankers were produced on pear and apple with both strains, the virulence of the two on all the common fruit trees being about equal. The amount of infection seemed to vary more with the condition than with the kind of tree; succulent, rapidly-growing bark being much more susceptible.

NATURAL INFECTION IN NURSERY TREES FROM 1915 TO 1920

From 1915 to 1920, occasional lots of nursery stock were found to be infected with the cankers, but the loss was never serious or alarming. The disease in most cases was evidently contracted during heeling in, judging from the fact that the cankers were scattered along the entire length of the tree and mostly on one side. There was little opportunity, however, to trace the matter to its source until the winter of 1921. This was a season of excessive rainfall in California, beginning early in November. During January and February there was a very heavy loss from this disease in nursery stock, particularly in the Sacramento Valley where it amounted to hundreds of thousands of dollars within a few weeks. Perhaps the greatest damage was in apricots and peaches, although almonds and plums, as well as apples and pears, were almost equally involved. It was evident at this time that much infection had occurred in the nursery row before the trees were dug, especially where they were small and thickly set, from splashing up of the surface soil. June-budded apricots and peaches were the worst infected, practically every tree in large blocks being cankered just above the bud. Four or five cankers to a tree were a common occurrence. The heaviest loss occurred in trees heeled in in exposed trenches. Isolations were readily made from such cankers while the bark was fresh; and by taking from the wood underlying the outer edge of the canker, cultures have often been secured after the bark was badly dried. Both strains A and B were found in such material, but the effect produced was the same in all cases.

INFECTION IN BEARING PRUNE AND PEACH TREES

During 1921 and 1922 many large trees in full bearing were killed or badly injured by large cankers at the crown, often girdling the tree. The condition was similar to that often attributed to winter killing or sun scald, and was no doubt largely contributed to by the unusual extremes of temperature and moisture occurring during this period. In making cultures from such cankers, however, pythiaceous strains were isolated from prune and peach orchard trees from three to eight years of age. These cases were as follows: Prune tree about five years old from Butte County, characteristic of a general condition in the orchards of this district in the winter of 1921. The tree was in good condition the year before, but the foliage withered after starting to leaf out in the spring. More than fifty per cent of the trees in some orchards were in this condition. The canker, just above the crown, was mostly covered by heavy soil washed up by the rains. In the tree cultured the canker was five or eight inches wide and nearly girdled the tree. The bark was slightly sunken in the older parts of the canker, but aside from this and slight gumming the bark was fairly normal in appearance on the outside. On peeling away the outer bark, the affected area was seen to have much the same appearance as in Pythiacystis cankers on nursery trees, viz., a white, rather cheesy consistency in the central, older portion of the canker, the color turning darker with age, the border of the canker somewhat marked by zonate bands from light brown to translucent at the outer margin. The bark was affected down to the wood over most of the canker, which was active in spots at this time. Cultures were obtained in nutrient agar slants after twenty days. The fungus isolated, strain C, is discussed elsewhere. We have had better success in isolating the fungus from old, dried-out cankers by planting in agar slants than by any other method, as in many cases it pushes down and beyond the surface growth of other organisms.

In the latter part of December, 1920, a peach tree five or six years old showing crown canker of a similar nature was also cultured with positive results. This tree was obtained from Placerville and represented a common condition at the time in that region. The stem was about 2½ inches in diameter at the point of infection. The canker was mostly on one side and seemed to be of long standing. The lower portion was already invaded by various saprophytes, but the upper margin was still actively pushing out and showing the characteristic zonation. From this tissue cultures of the fungus were readily obtained, as described elsewhere under strain D.

Following this experience, an effort was made to determine the occurrence and seriousness of this infection in deciduous fruit trees of all ages.

INOCULATIONS IN BEARING TREES

On January 27, 1922, the following inoculations were made in Berkeley trees. 1. A cherry tree (*Prunus avium*) eight or ten years old, perfectly sound and growing rapidly, trunk 15 inches in circumference. 2. Apricot (*Prunus armeniaca*) eight or ten years old, a large tree, but with bark more or less sunburned. 3. Plum tree (*Prunus domestica*) 13 inches circumference. 4. Apricot, badly sunburned, 12 inches circumference. 5. Apricot tree 9 inches circumference, trunk in poor condition.

Tree 1 Inoculated with strain C, from bearing prune tree.
" 2 inoculated with strain A, from peach nursery tree.
" 3 inoculated with strain C.
" 4 inoculated with strain D, from bearing peach tree.
" 5 inoculated with strain D.

The inoculum was taken from potato agar cultures five days old, all showing abundant growth on the surface. The trunks were first sponged with formaldehyde, then with sterile water. Two or three stabs were then made in the bark just above the rootstock on the north side of the tree, using sterile scalpels pointed downward at an abrupt angle, the cut extending to the wood. Considerable mycelium was then inserted into each cut on the point of a sterile needle, tucking it well into the cut. Check cuts were made on each tree a little to the right and facing the north side, the inoculations to the left, with three or four inches between. The inoculations were not bound or covered in any way. Soil from another part of the yard

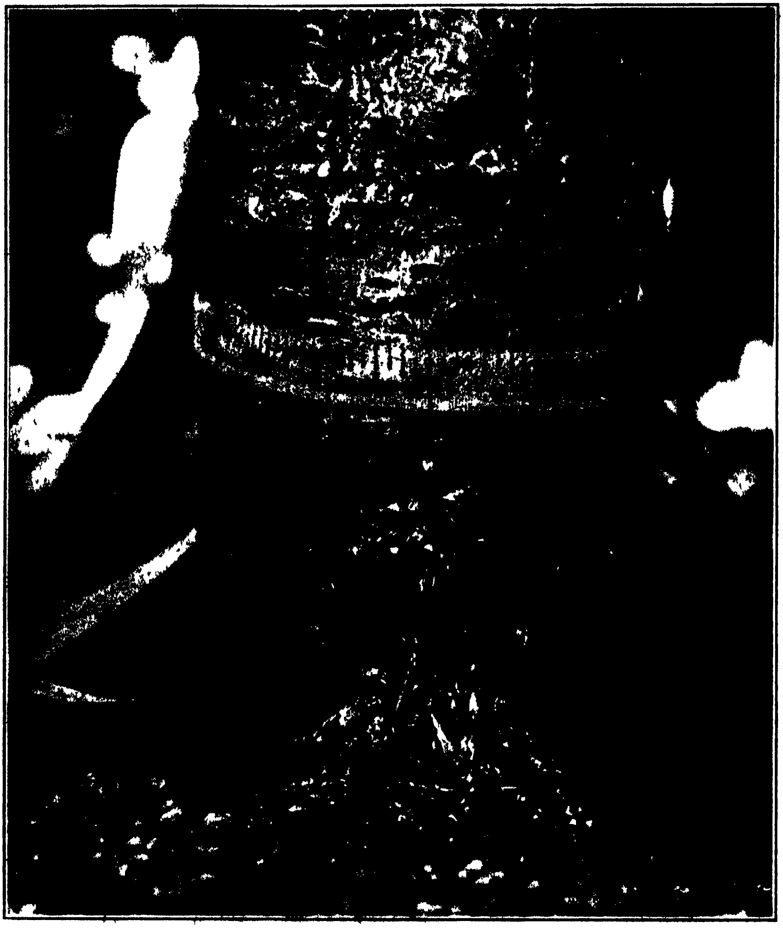


FIG. 2. Lesion produced on sweet cherry tree by inoculation with strain C.

was piled rather high about the tree without coming into actual contact with the cuts. Two days later, rain and snow fell for about twelve hours. The rainfall for the period was rather moderate after the middle of February.

Results on May 24, 1922. On tree 2 the fungus had failed to grow and the cuts were practically healed. Trees 3, 4, and 5 showed small cankers. All were gumming considerably and showed the characteristic tissue discoloration. On tree No. 1, under closer observation on account of its better condition, the canker was found to be progressing more rapidly with profused gumming, as shown by figures 2 and 3. The fungus was recovered from the cankers in Nos. 1, 4, and 5 only. The checks showed no infection in any case. At the end of this period the cankers were cut out and the wounds treated.

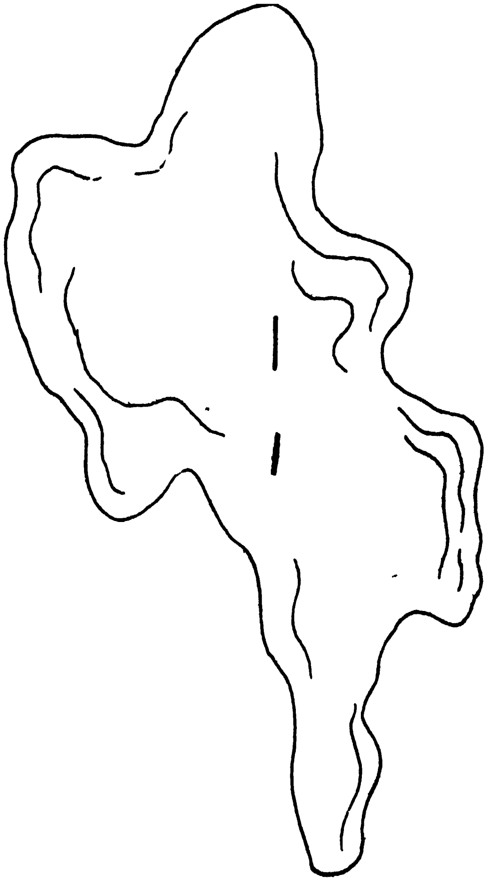


FIG. 3. Outline of lesion produced on sweet cherry tree in four months by inoculation with strain C.

INOCULATIONS AT MOUNTAIN VIEW

On February 2, 1922, the following trees were inoculated by Mr. Bert Rudolph, at Mountain View, Santa Clara County, using the same strains: three mature apricot trees, one with each strain; two-year-old trees of plum and peach in orchard form. In this experiment the inoculum was inserted under flap cuts made just above the crown, then covered with a sterile linen pad. These trees were destroyed before final records were made. When observed on March 10, the two-year trees were gumming profusely with every indication of active infection. The inoculations on older trees were not successful.

WALNUT CROWN CANKER OR ROOT ROT

In the spring of 1923 several specimens of crown canker or root rot of northern California black walnut trees (*Juglans hindsii* Jepson) were cultured for Pythiacystis, and from one of these a culture was obtained in nutrient agar slant. This work was done in May and June, very late for active infection, and with old cankers nearly girdling the tree. By cutting down to the wood, however, the fungus was secured after the tissue had been nearly a month in culture. It appears from our experience with larger trees that while the organism may die out during the summer throughout a large part of the canker, it lives over at scattered points along the margin in the outer wood layer, where it seems to find some protection. In this way cultures have often been secured from nursery trees in a very dry condition. The walnut canker was of the same general type described for bearing peach and prune. A number of trees affected in this way have been seen in San Joaquin County, where this root rot or canker constitutes rather a serious disease.

Walnut Inoculation. Owing to the dry winter of 1923–24, no inoculations were tried until December, 1924. Two trees were then inoculated at the crown, as described for other strains. One of these trees was a black walnut seedling, the other a grafted tree of unknown variety. Small cankers about two inches in diameter have developed to date.

DISCUSSION OF THE MORPHOLOGY OF FIVE STRAINS WHICH HAVE BEEN TESTED BY INOCULATION

A. Original nursery stock strain. This differs in no important character from the fungus causing brown rot of lemon and described by the writers as *Pythiacystis citrophthora.* No oospores or chlamydospores have been developed. Growth on agar media is almost entirely submerged and the production of conidia usually very scanty. Abundant sporangia are developed in liquid culture as it dries down along the edge. Sporangia are extremely variable as to size and shape. We may mention here an isolation from peach nursery stock showing typically blunted sporangia as in *Phytophthora syringae,* but no oospores.

B. From almond and other nursery stock infections. A strain similar to *P. citrophthora,* but producing abundant oogonia and antheridia in potato agar. The oospores average 30–40 µ, running up to 44 µ in diameter, thick-walled, with both paragynous and amphigenous antheridia.

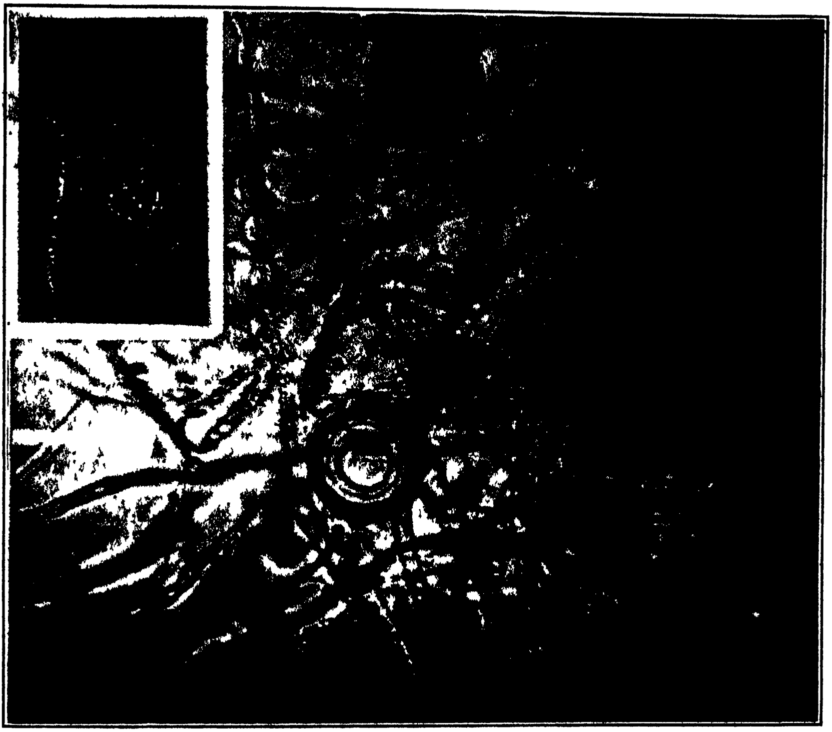


FIG. 4. Oospores of strain B. Inset shows individual with amphigenous antheridium.

C. From crown canker of bearing prune tree. While oogonia are always present, sporangia are comparatively rare. In potato and nutrient agar the mycelium develops a heavy, white, aerial growth an inch or more above the medium and penetrates somewhat below the surface. The oogonia are scattered thickly through the lower portion of the aerial mycelium, which is straight, coarse, not profusely branched. The oogonia are small, rather thick-walled, developed on slender stalks. The average size is about 16–18 µ in potato agar. The antheridia are small, club-shaped, slender stalked, and may meet the oogonium at any point on its surface. The strain develops occasional bodies slightly larger than the usual oospore, with thick walls showing distinct radial striations. These may be oospores, but their connection with an antheridium has never been observed. The sporangia from this strain, produced in one instance only by drying down liquid cultures, are lemon-shaped, averaging about 16 µ in length, with distinct papilla.

D. From crown canker of bearing peach. This strain has considerable resemblance to strain E from walnut in that abundant sporangia are produced on the surface of potato agar cultures after three or four days, the mycelium becoming distinctly tuberculate and growing only slightly above the medium. So far, however, it seems to differ from strain E in that oospores are scarce in potato agar, perhaps one being found in several mounts, widely scattered among the sporangia. The antheridia are both paragynous and distinctly amphigenous. Sporangia are almost spherical in most cases, with prominent papilla, this being the most striking characteristic of the strain. The average diameter of the sporangia is about 30–42 µ, although many are smaller. The average diameter of oospores is about 28–34 µ. This strain develops abundant chlamydospores on potato agar after three or four weeks.

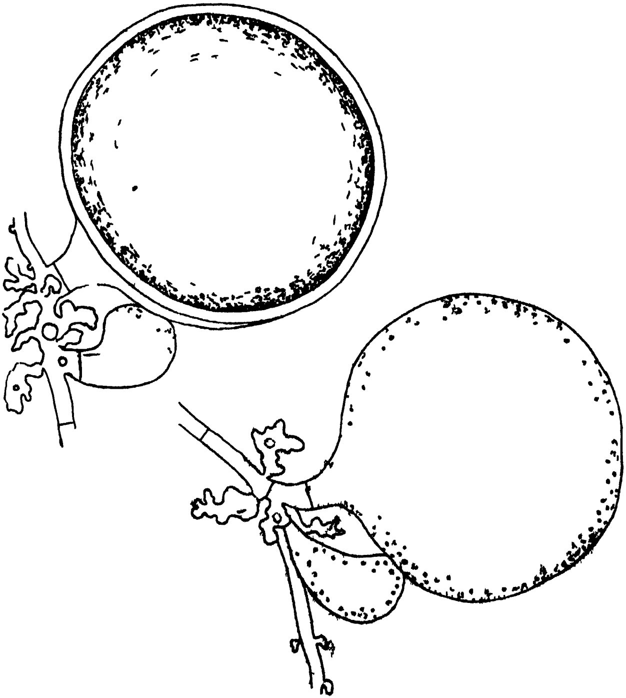


FIG. 4. Oogonia and antheridia of strain E from walnut.

E. From crown canker of black walnut. The antheridia of strain E are prominent and are mostly paragynous, although occasionally they appear to surround the oogonial stalk. The base of the antheridial stalk seems typically very near that of the oogonium, and in most cases the antheridium comes in contact with the oogonium just to one side of its base, as in figure 5. The latter characteristic is described by Rose (54) for his strawberry strains. On potato agar a scanty aerial mycelium, about 5 mm above the medium, quickly develops tuberculate masses, as described by Rosenbaum (30) for *P. syringae*. The tuberculate mass usually surrounding the oogonium is figured in its simplest form and in cross section The readiness with which fruiting bodies are produced is extremely variable, but usually when inoculum from an old culture is planted in fresh potato glucose agar, an abundance of both oogonia and sporangia is quickly formed on the surface (Fig 6). The sporangia separate very readily from the stalk at the cross wall just below the base, as in *P infestans* and other species. In fact, it is often difficult to find the smaller ones still attached. The oospores average from 25 to 30 μ in diameter. The sporangia are exceedingly variable in size and shape. Of the larger sizes, about 25 x 46 μ is most common, tapering from the base; although a more slender lemon shape, about 27 x 43 μ, is abundant. Of the small sizes, which are more numerous, a broad oval to nearly circular is a common type, the average length about 25 μ. Many of these have the papilla on one side.

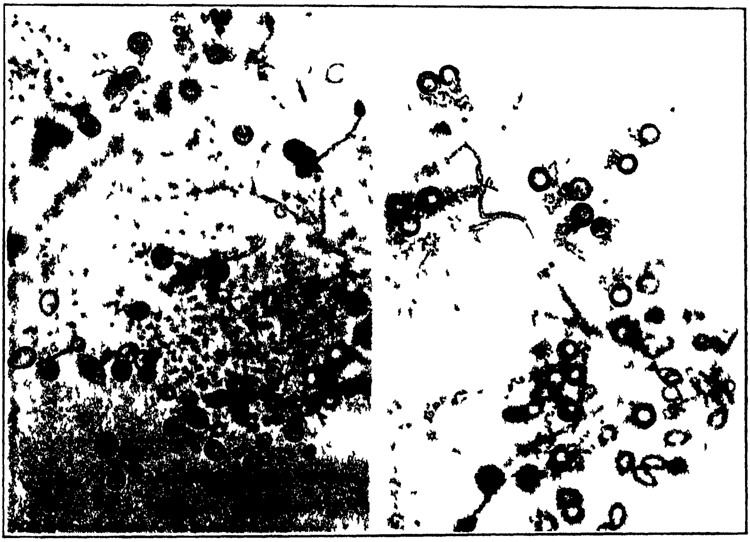


FIG 6 Oospores and conidia of strain E from same culture.

Both the peach and walnut strains (D and E) seem to be typical of *Phytophthora cactorum,* although strain D has produced very few oogonia. The prune strain (C) is of the cactorum group according to Rosenbaum, but has the general character of the genus *Pythium,* as now understood.

Under strain A, from nursery stock (considered as typical *Pythiacystis citrophthora*), have been placed all forms developing sporangia only and these sparsely on potato agar but rather abundantly at the edge of liquid media. Some of the isolations show the lemon-shaped conidia typical of *P. citrophthora,* others distinctly pear-shaped as in *Phytophthora syringae.* The mycelium in the host plant is entirely sterile.

TAXONOMY

The comparative study of these and other strains of this group over a period of several years, together with consideration of the literature, creates a strong doubt as to the validity of the present distinctions between the genera *Pythium, Phytophthora,* and *Pythiacystis.* Fitzpatrick (49) has proposed merging *Phytophthora* with *Pythium* on account of the occurrence of species of the latter in which both the characteristic types of zoospore formation occur—*i.e.,* differentiation within the sporangium and that outside in a vesicle. The latter is the only definite morphological character on which *Pythium* is based. Species of *Phytophthora* are also known in which a thin-walled vesicle is formed at the mouth of the sporangium (Rosenbaum, 30). The evanescent, colorless texture of the sac renders observation of this in both genera difficult and inadequate. It is particularly evident in our walnut strain that such a membrane exists. Sporangia of a slow-growing culture, as on a glass slide in a moist chamber, are good for such study. Here, after several days in scant moisture, sporangia are often found, the contents of which had begun to emerge, then receded back into the sporangium. In such cases a distinct sac may be clearly seen at various initial stages extending out as a stretched lining from the ends of the sporangium wall to the tip. It is also true that the matter of habitat, once considered of some importance in distinguishing *Pythium* from *Phytophthora,* is now valueless, as so many soil organisms have been placed in the latter genus.

We have already discussed reasons for combining *Pythiacystis* with *Phytophthora,* based on the abundance of soil-inhabiting species and strains of the latter which are now known. We have, therefore, at present a series of species and strains intermediate between all three of these genera and between which no sharp distinction can be drawn upon the basis of the features which formerly were considered characteristic. It seems to us that the time is not yet ripe for deciding whether *Pythiacystis* should be merged with *Pythium* or with *Phytophthora,* or whether all three should be merged together, or an entirely new sub-grouping be established. This is on account of the fact that there is so much variation in size, form, type, and occurrence of reproductive bodies and that so many intermediate forms are continually being discovered. A restricted genus *Phytophthora* based on the original type, *P. infestans* de Bary, with quite definite conidiophores and typical aerial, parasitic habitat would be fairly logical at present. This, of course, comes back to the original distinction between *Phytophthora* and *Pythiacystis*. As suggested by Fitzpatrick (49), the name *Pythium* has precedence over *Phytophthora* and should be retained in event of combining the genera under consideration.

SUMMARY

1. Since the genus *Pythiacystis* was described by the writers in 1906, several new species of *Phytophthora* have been described, representing types which obscure the distinction between these genera.

2. These species are characterized by the occurrence of a variety of strains and morphological variations which make specific and sub-generic delimitation somewhat uncertain.

3. A number of pathogenic strains have been found in northern California which form a closely connecting series between typical *Pythiacystis citrophthora* and fungi of the *Phytophthora cactorum* type.

4. A brief description of five of these strains is given. Four of them resemble *Phytophthora cactorum*, with paragynous antheridia, and in three of these the amphigenous type is also found. The fifth strain is similar to typical *Pythiacystis citrophthora*, having conidia only. None of these strains is selective as to host, but all seem to produce about the same symptoms.

5. Fungi of this type cause crown or trunk canker in nursery and orchard trees of pear, peach, almond, apricot, cherry, plum, prune, and black walnut. A great deal of damage is done to nursery trees by diseases of this sort, particularly in wet seasons.

6. In regard to taxonomy, various forms described by the writers and others appear to obscure the present distinction between the genera *Pythium* Pringsheim, *Phytophthora* de Bary, and *Phythiacystis* Smith and Smith. If these genera are merged, the name *Pythium* would have precedence.

7. The changing of *Pythiacystis* to either *Phytophthora* or *Pythium* is not proposed at present owing to the doubtful status of the delimitation of these genera.

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SPRAY INJURY TO APPLE

H. C. Young and R. C. Walton

With Plate XIV

The data here presented were secured in connection with an investigation of the fungicidal properties of sulphur. This investigation was conducted under the direction of the Crop Protection Institute in administering a research fund provided jointly by the Freeport Sulphur Company, the Texas Gulf Sulphur Company, and the Union Sulphur Company.

The studies here recorded were made possible through the generous cooperation of the New York, Geneva, Agricultural Experiment Station and especially through the personal assistance of Dr. R. W. Thatcher, Director, and Professor P. J. Parrott, Chief in Entomology.

The injury to foliage and fruit resulting from spraying during the season of 1923 was exceptionally serious in many localities. Severe cases of such injury have occurred occasionally since spraying began. This has been true when Bordeaux mixture and lime-sulphur were used, but rarely have the wettable and "dry mix" sulphurs and sulphur dusts caused injury as severe as during the past season. This was especially true in the states of New York and Pennsylvania where, in general, the weather conditions were not extreme, except for one week of very hot weather in June. On the whole, the season from June to August, inclusive, was quite dry. During late July injury was observed following the use of many kinds of spray materials. At this time an effort was made to determine the types of spray injury to fruit and leaves and the underlying cause or causes of such injury.

Several attempts have been made to determine the cause of spray injury, but because of the complex chemical changes that take place in spray mixtures both before and after application and the various climatic conditions that affect these changes, little definite progress has been made. The physiological condition of the tree seems also to be an important factor.

One of the first comprehensive studies of spray injury was made by Wallace (9). He diagnosed several types of injury the most common of which was tip burning. This, he states, results from the accumulation of the spray liquid at the tip. Other types noted were: (1) injury following fungus infection; (2) the scorching of large parts of the leaf surface, which results when foliage is drenched; (3) Bordeaux injury, which may appear at first as very small spots gradually increasing in size. It is the opinion of the author cited that the action of lime-sulphur and Bordeaux are different, the action of the former being immediate, that is, within three or four days, while that of the Bordeaux is more moderate at first but extends over a much longer period of time. The author concludes that there is no correlation between the weather at the time of, or after application and the resulting injury; that possibly the weather conditions previous to the time of application may so influence the foliage as to make it more or less susceptible to lime-sulphur spray injury; that the agent active in causing lime-sulphur injury is doubtless the soluble sulphur in the form of a calcium sulphide.

Safro (5) is of the opinion that the concentration of the soluble sulphides of lime-sulphur is the important factor in burning. He states that the only ingredients in lime-sulphur that are instrumental in burning are the polysulphides and thiosulphate. He states that the specific gravity of any lime-sulphur solution is not an index to the amount of burning that can be expected. "Though the amount of soluble sulphides in the spray may be known, other factors still remain to modify, sometimes radically, the power of a spray to produce injury to foliage or fruit."

In 1912, Stewart (7) studied sulphur-arsenical spray injury. He states that the chief factors concerned in this type of spray injury are: (1) the character of the arsenical, (2) the purity of the sulphur solution, (3) the soundness of the epidermis on foliage and fruit, *i.e.*, its freedom from punctures and breaks due to insects or fungous attacks, (4) density and abundance of the application, (5) the size of the drops that collect, and the time required for their evaporation, (6) differing resistance of varieties, (7) excessive temperatures, (8) general weather conditions. The best method that he found for eliminating injury was by using the ortho-arsenate of lead which is the tri-plumbic arsenate, Pb_3 (As O_4). The pyro, Pb_2 As_2 O_7, and acid arsenate, Pb H As O_4, are stable only in acid mixtures while the ortho is more stable under neutral and alkaline conditions. The addition of sugar of lead frequently reduces burning in arsenical lime-sulphur mixtures.

Patten and O'Meara (4) studied injuries following the use of calcium and magnesium arsenates.

Frequently burning of foliage can be traced directly to the arsenicals. Fernald and Bourn (2) studied arsenical burning and concluded that neutral lead arsenate was the safest material, especially in clear weather; that clear weather spraying is safer than the cloudy weather; that spraying can be done safely at high temperatures if the humidity is low; that spraying can be done when the humidity is high if the temperature is low. Krout (3) also attributes much to the weather and states that apples should never be sprayed when the temperature and humidity are both high, as burning of foliage is almost certain to result. He states further that sulphur dusts have never burned the foliage, while burning with copper-lime dust is frequent.

Investigations extending through a period of 10 years and involving an application of various arsenical mixtures to 10,000 separate plants have been reported by Swingle, Morris and Burke (8). They attributed most of the injury resulting from spray mixtures containing arsenicals to the soluble arsenic. They have found it quite difficult to make exact statements concerning the toxicity of the different arsenicals because of the variability of their composition. One might theoretically expect ortho, meta and pyro arsenates, each in the form of monoplumbic, diplumbic and triplumbic salts, or in other words, a total of 9 possibilities. While this is not actually possible yet commercial lead arsenates are not single salts of lead and arsenic acid but a mixture of different lead arsenates. A company's product may change within a short period, even that prepared at the same factory. These authors conclude that arsenic trioxid, which was formerly thought to burn in all concentrations, is not so dangerous, provided it is applied promptly after mixing with water. Leaves injured by arsenicals show evidence of this by turning brown. They state that the injury to foliage is practically through the lower epidermis, regardless of the number of stomata in the two surfaces. This injury is influenced more by humidity than by temperature.

In this short review of work on spray injury no very definite conclusion can be obtained. The indications are that much of the injury resulting from lime sulphur-lead arsenate mixtures is due to the soluble arsenicals, but not all may be. Lime-sulphur alone will burn foliage. Bordeaux mixture alone will cause severe burning. It is to be admitted that climatic factors play an important rôle. In determining the underlying cause of injury not only the above factors must be considered, but in addition, the chemical changes taking place before and after application of any mixture must be considered. The question to be answered is why, under certain conditions, do many sprays burn foliage and russet fruit.

The work reported in this paper was begun in the latter part of July, 1923, when injury to fruit and foliage was at its height in New York and Pennsylvania. It was realized that many points could not be determined because of the lateness of the season. The report contained herein gives a summary of the types of injury induced by the various spray mixtures and the results of some experiments on the chemical compounds thought to be responsible for injury. The experiments were conducted in several orchards in the vicinity of Geneva, New York, and in Adams County, Pennsylvania.

TYPES OF INJURY

I. *Edge burn* was the most common type of injury found. It is characterized by injury to the edge of the leaf, which parts turn brown and may finally slough off. Various degrees of injury result, from a small area to the entire leaf edge. Frequently only a small portion of the leaf surface remains on each side of the mid rib. This type of injury does not appear to be correlated with high temperatures. It is as severe on one side of the tree as the other, that is, relative to north, south, east or west. It appears to be more severe in the paths of the spray gun. It occurred alike on all the varieties tested, namely, Greening, Rome Beauty, Winesap, Stayman Winesap, York Imperial, Northern Spy, Baldwin, Black Twig, King, Wealthy and Dutchess. The injury appears within three or four days after application of spray. It is not confined to the use of lime-sulphur, but occurs in somewhat less degree and in modified form with colloidal sulphur and slightly with precipitated sulphur. With these sprays and with dry mix the injury largely takes the form of a moderate tip burn. This type is shown in plate XIV, D, and a record of occurrence is given in table 1.

II. *Scald* is a type of spray injury found on both foliage and fruit following the use of various sulphur sprays applied in extremely hot weather. Scald on the foliage differs from edge burn in that usually larger areas of the leaf are involved and very frequently the entire leaf. The areas are, as a rule, lighter brown than the edge burn injuries and are not in the form of small spots, as is the case with Bordeaux injury, but are large. They may extend to the margin of the leaf or may involve the entire surface.

On fruit the scalded areas are light brown in color with irregular margins. They may be largely superficial and may callous over and be scarcely noticeable at picking time. Seriously injured areas have a more definite margin, are darker in color and may involve the skin and part of the flesh beneath. The injury on Stayman Winesap appeared quite different from that on Rome Beauty.

Both the foliage and the fruit types of scald are found much more abundantly on the lower half of the trees. Whether this is due to greater force of application from the spray nozzles or to a greater amount of spray material on the fruit and foliage is not known. Scald appears to be correlated with extremely high temperatures. It is always found on the southern exposures of the trees and never on the northern, unless an apple or leaf is so located as to receive the direct rays of the sun. Various sulphur sprays will produce it, but in the cases observed in 1923 it was much more serious on trees sprayed with commercial lime-sulphur. Dry mix, colloidal and precipitated sulphurs produced it, but only to a slight extent. 90–10 dust produced it and pure sulphur dust caused it during the season of 1923, if the trees were dusted when the temperature was around 100° F. in the shade. There does not seem to be any considerable difference in susceptibility among varieties. It appears probable that scald is a sulphur burn and its severity is correlated with temperature conditions. Bordeaux mixture did not cause it in 1923, whereas lime-sulphur applied the same day in the same orchard produced serious injury. Arsenate of lead was used in both materials. Scalded leaves and fruit are shown in plate XIV, A, B, and C, and the relative occurrence is indicated in table 1.

III. *Yellow leaf* is a further type of spray injury. It appears within a week or ten days after lime-sulphur has been applied in late July and August. This type of spray injury is characterized by the leaves turning yellow or partially yellow and falling off. A greenish and yellowish mottling is characteristic. In some orchards the majority of the yellow leaves are the smaller, older ones which appear early in the spring around the blossom clusters. In other orchards the reverse has been noted, the yellow leaves being mainly the later, larger ones. One fact stood out prominently in 1923 in Adams County, Pennsylvania, namely, that the tip leaves were able to withstand this type of spray injury. Seldom were the tip leaves affected. Varieties differ in their susceptibility to this type of injury. In New York State the variety most severely affected when lime-sulphur was used was the King. In this case the early August spray caused 60 per cent of the leaves to fall off. Greening, Northern Spy and Baldwin varieties were less severely injured, approximately 15 per cent of their leaves falling after this spray. No yellow leaves appeared on the Rome Beauty when lime-sulphur was used. The same varietal susceptibility was noted in Adams County, Pa. The York Imperial was seriously affected, also Grimes Golden and Delicious, while the Stayman Winesap and Black Twig showed very little yellow leaf following any of the sulphur sprays used or following Bordeaux. In some cases varieties resisted lime-sulphur but did not the colloidal or precipitated sulphurs. This was the case with Rome Beauty in New York State.

Yellow leaf is, in the main, a mid-summer spray injury. It has been pronounced in southern Pennsylvania during the summers of 1922 and 1923, both of which were unusually dry. The same was true for New York conditions. It is not known whether the drought was a contributing factor, but it seems possible that results noted would be more likely to occur in dry weather than in seasons of normal precipitation. More definite observations are contemplated in an attempt to correlate weather conditions with this type of injury. Yellow leaf is frequently attributed to frost injury. This is not thought to be the case, although frost injured leaves are frequently the first to become affected. Leaves injured by frost are always puckered and when a leaf in this condition is bent the epidermis breaks so that the bright green tissues beneath are exposed. Yellow leaf is shown in plate XIV, E, and the prevalence of this type of injury with various spray materials is set forth in table 1.

IV. *Goose neck.* Goose neck is a term which was given by Dr. Waite, of the Bureau of Plant Industry, U. S. D. A., to a type of injury. This type is characterized by a longitudinal arching of the leaf, sometimes barely noticeable and at other times so pronounced as to produce a rolling.

Leaves but slightly arched frequently show no necrotic areas or other apparent injury, or at most, only a very slight amount. Severe arching is very frequently accompanied by more or less serious edge burn indicating that possibly the edge burn type of spray injury can be responsible for goose neck. However, this is certainly not always true because cases have been noted in New York and Pennsylvania where severe arching occurred on check trees. These trees were severely infested with the Red Bug and the scars produced by this insect were especially numerous on the goose neck leaves. Arched leaves frequently show slight injury to the mid-rib and it is thought that different kinds of injury may be responsible for this type.

Various types of goose neck are shown in Fig. 1, and the amount of this type of injury is indicated in table 1.

V. *Injury Following Apple Scab.* This is a type which has been recognized for years but about which little seems to be known and little has been written. It is characterized by a solid circle of light brown, necrotic leaf tissue in the center of which is the dark scab spot. The entire area looks not unlike a large frog-eye spot after secondary growth has taken place, with the exception, however, that the light center, so characteristic of the frog-eye spot, is lacking and in its place is the larger and darker scab area (Plate XIV, F).

The writers have no authentic data as to the cause of such injury. In 1923, it was quite abundant where certain sprays were used. For instance, in plots which received barium sulphide and arsenate of lead, both in New York and Pennsylvania, such injury was abundant. In another Pennsylvania orchard of Stayman Winesap it was very plentiful in a plot which received arsenate of lead only, up to and including the 10-day spray. In an adjoining plot which received lime-sulphur only, up to and including the 10-day spray, there was practically no injury of this type. Both plots were treated alike after the 10-day spray, receiving one application of sulphur dust. In this orchard, the indications are that the arsenate of lead is responsible but this cannot be definitely stated until further work has been done.

VI. *Leaf roll* is thought by some to be a type of spray injury. This is characterized by the margins of the leaf rolling upward. The authors could collect no data which would lead them to believe that it was a result of spraying.

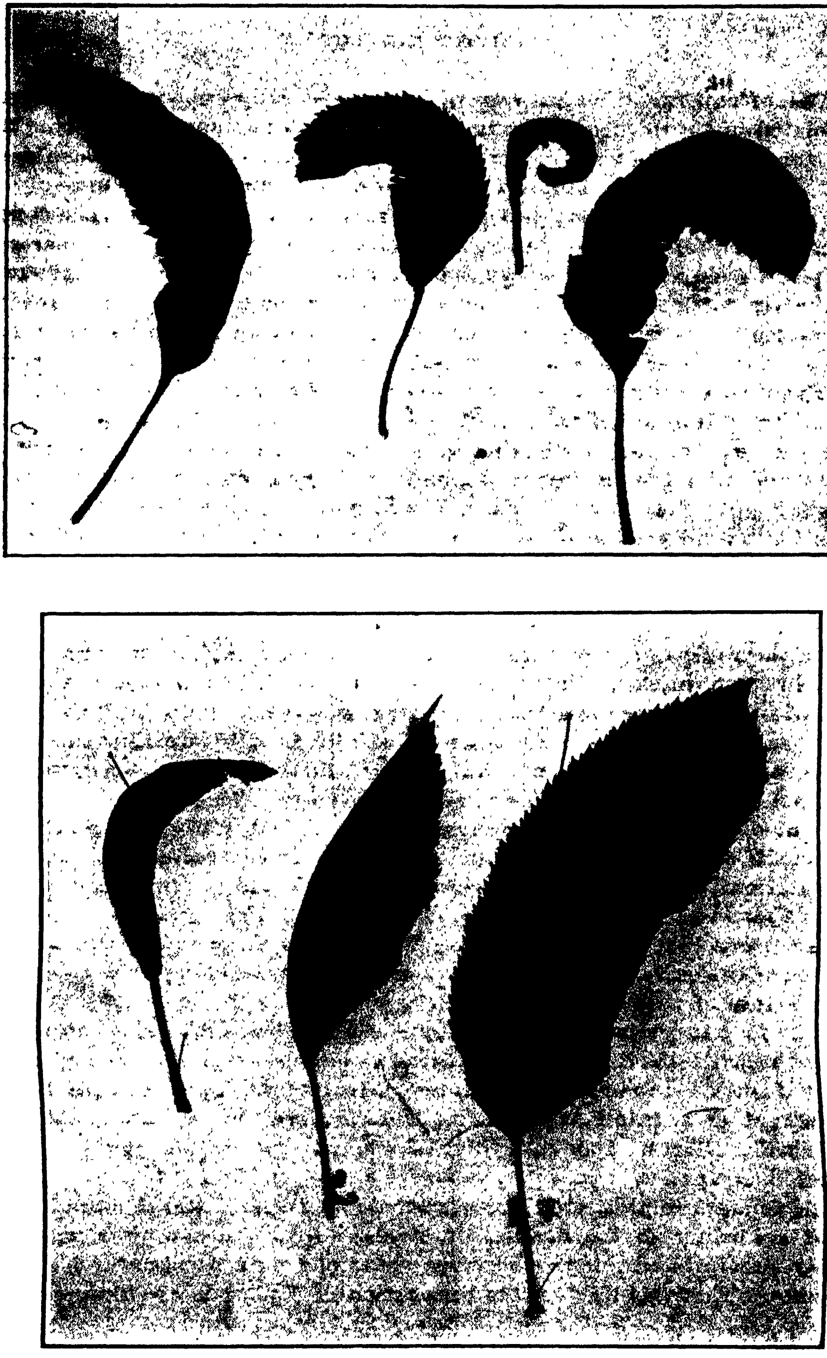

FIG. 1. Various types of goose neck.

VII. *Fruit russeting* is a type of injury generally known. It is usually associated with Bordeaux mixture or other copper sprays. During the summer of 1923 the writers became convinced that lime-sulphur can cause it. In Adams County, Pennsylvania, plots were sprayed with lime-sulphur and Bordeaux mixture, leaving one unsprayed plot as a check. The copper plots showed 50 per cent russet, the lime-sulphur plots 15 to 31 per cent and the check plots only a very small amount. In New York State plots of the Greening varieties were sprayed with lime-sulphur and Bordeaux. Lime-sulphur plots showed 10 per cent russet while Bordeaux showed 65 per cent.

Types of copper spray injury, other than those mentioned above, have been discussed by other workers. The authors observed that a long period may elapse after the application of a copper spray before the injury appears and that the injury may finally be much more severe than when sulphur sprays are used. One case was noted in New York State where pear trees were practically leafless two months after a mixture of copper and lime had been applied.

CAUSE OF SPRAY INJURY

In the review of literature it will be noted that various factors contributed to spray injury. There is no doubt in the minds of the authors that climatic factors are definitely correlated with some types of injury. In part the type of injury produced will be influenced by weather conditions. However, the weather alone will rarely cause burning. This must be induced by some ingredient in the spray mixture. This ingredient may be any one of many. It is not necessarily an arsenical. Most sprays that can be classed as fungicides may burn foliage under certain conditions. This was demonstrated during the season of 1923. If a spray has enough soluble material in it to kill fungi, it may burn foliage.

To prove that it is the soluble material in a fungicide that should be viewed with suspicion, a series of experiments were conducted with sulphur and certain of its compounds.

The first experiment was made by spraying commercial lime-sulphur on young peach foliage. Peach was selected because it is very susceptible to spray injury. Lime-sulphur, diluted 1 to 50, was applied with a hand sprayer at 11 A. M. on August 3rd. The temperature was about 88° F. in the shade. Severe foliage injury appeared within 36 hours, as was expected.

In contrast to this, trees were sprayed with precipitated sulphur prepared according to the method of Young (10). The sulphur was completely precipitated and the mixture contained only sulphur and calcium sulphate. This was used in the proportion of 5 pounds of sulphur to 100 gallons of spray mixture. This was the concentration used for apple spraying. Only slight injury to the foliage could be detected.

Another trial of the precipitated sulphur was made when conditions were thought to be more favorable for burning. At 1 o'clock on August 6th. two peach trees were heavily sprayed. The humidity and temperature were both high. That afternoon, about 4:30, it began to rain and continued to rain for an hour or more. The following day the sun was bright and temperature quite high. Only slight injury followed.

A number of experiments were conducted with colloidal sulphur prepared in various ways. The initial ingredients were sodium thiosulphate and sulphuric acid. When these are mixed colloidal sulphur is produced, also sodium sulphate and sodium thiosulphate. In one test the colloidal sulphur was centrifuged out and washed four times with distilled water. A mixture of this was sprayed on beans, potatoes, rose and tomato leaves. No burning resulted. Similarly colloidal sulphur washed one, two and three times was used. Burning on roses, tomatoes and beans was greatest with the material washed the fewest times. Separate experiments were made using sodium sulphate and thiosulphate with the result that each burned in concentration that was not toxic to fungi.

In the course of summer field work, when the colloidal sulphur was prepared on a large scale, it was impossible, because of the limitations of apparatus available, to free it of these soluble materials. There resulted frequent injury from its use on apple, and this injury was most severe on peach leaves.

DISCUSSION AND RESULTS

In our work evidence points to the soluble material in spray mixtures as being directly responsible for the burning of leaves and russeting of fruit. A spray mixture may contain one to many soluble compounds. Some of these soluble compounds are electrolytes of strong coagulating power. When such compounds come in contact with the protoplasms of the very active leaf cells injury may result, probably through plasmolysis and coagulation. This is particularly evidenced when the leaf is previously injured by fungi, thereby permitting the soluble fungicide to diffuse through the dead tissue to the living tissue.

When sprays are applied with a gun so that the mixture strikes the leaves with considerable force injury may result. Possibly the lower epidermis is weakened or injured and the cuticle partially penetrated by such force. This is not a new observation but has been noted ever since spraying began. If the spraying is not carefully done it often happens that one can tell where the spray nozzle or gun was located during spraying because of the paths of injury.

TABLE 1.—*Types of spray injury at Geneva, N. Y., caused by various spray materials; results expressed as percentages*

Kind of tree	Type of spray	Free from injury	Duplication	Edge burn			Goose neck with injury		Goose neck without injury		Scalding	Injury following scab	Yellow leaf	Total No. of leaves
				slight	mod.	sev.	slight	sev.	slight	sev.				
	Geneva Experiment Station Orchard													
Rome Beauty	Lime-sulphur	14.1	9.4	31.2	28.5	19.1		12.6			3.3	0.1	0.3	1,000
	Lime-sulphur and casein	14.0	16.4	38.2	29.4	17.4	3.6	8.4	0.4		7.7	0.0	0.9	1,000
	Sulphur glue spray 8–4–50	94.2	0.6	1.8	1.8	0.0	0.6	0.0	0.6	0.8	0.8	0.0	0.0	500
	Sulphur lead arsenate dust 90–10	93.6	0.0	2.4	1.4	0.2	0.8	0.2	0.8	0.0	0.8	1.4	0.0	500
	Precipitated sulphur	87.2	1.8	6.6	1.8	0.4	0.8	0.6	0.0	0.0	2.8	1.2	0.4	500
	Colloidal sulphur	41.6	16.9	28.5	15.9	9.9	3.8				1.9	0.37	14.6	1,100
	Wilson Orchard													
Greening	Lime-sulphur	35.3	19.5	47.1	6.3	1.2	0.4	1.1	1.8	0.1	0.7	0.0	15.4	1,000
	Lime-sulphur and casein, 1 lb. to 100	38.0	21.0	47.3	4.7	0.8	1.2	0.7	2.2	0.6	3.3	0.0	16.2	1,000
	Lime-sulphur and casein, 2 lbs. to 100	31.0	20.8	50.0	9.1	3.8	0.6	1.0	2.4	0.2	2.8	0.0	17.4	1,000
	Lime-sulphur and 1–40 glue	28.3	30.2	52.3	5.3	1.7	1.7	1.0	4.1	1.1	5.1	0.0	22.0	1,000
	Sublimed sulphur, No. 8, and casein spreader	88.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.4	0.4	0.0	0.0	500
	Same and 16 lbs. lime	82.2	0.2	0.0	0.0	0.0	0.0	0.0	5.2	0.2	0.0	0.0	0.0	500
	Precipitated sulphur	59.6	11.9	29.5	0.6	0.3	0.5	0.1	0.9		0.1	0.0	14.3	1,000
	Colloidal sulphur	47.0	11.4	41.8	5.2	2.0	0.5	0.0	0.1	0.1	0.1	0.0	12.5	1,000
	90–10 dust, regular schedule	81.4	0.2	0.0	0.0	0.0	0.0	0.0	4.2	0.2	0.0	0.0	0.0	500
	Check	84.2	0.0	0.0	0.0	0.0	0.0	0.0	2.4	0.0	0.0	0.0	0.0	500
	Post Orchard													
N. Spy	Lime-sulphur	41.8	12.9	44.1	6.1	1.1	1.1	0.7	0.0	0.0	1.5	0.0	16.4	1,000

Climatic factors influence the chemical activity of spray mixtures. Spray materials containing some form of sulphur which must be acted upon by climatic factors before it becomes fungicidal, rarely burns severely when used alone. Sulphur is believed to be toxic because of its oxidation product and this product is formed but slowly at ordinary temperatures. It is possible that at extremely high temperatures sulphur may burn, but such cases are rare. The effect of temperature on the chemical action of soluble materials is necessarily an important one. Undoubtedly the action is accelerated both in a chemical and physical manner.

Climatic factors undoubtedly can influence the burning action of lime-sulphur. This solution is extremely alkaline and remains so until the mixture is dry or acted upon by the CO_2 of the air. The complete change of lime-sulphur to precipitated sulphur and calcium sulphate requires from one-half to two hours. If this process is retarded and if conditions are favorable, the soluble sulphides may burn.

It is to be expected that the various soluble chemical substances contained in spray mixtures would cause different types of injury. It is the opinion of the authors that a sufficiently definite study of spray injury can be achieved only through correlating climatic records with a study of the soluble compounds contained in spray mixtures.

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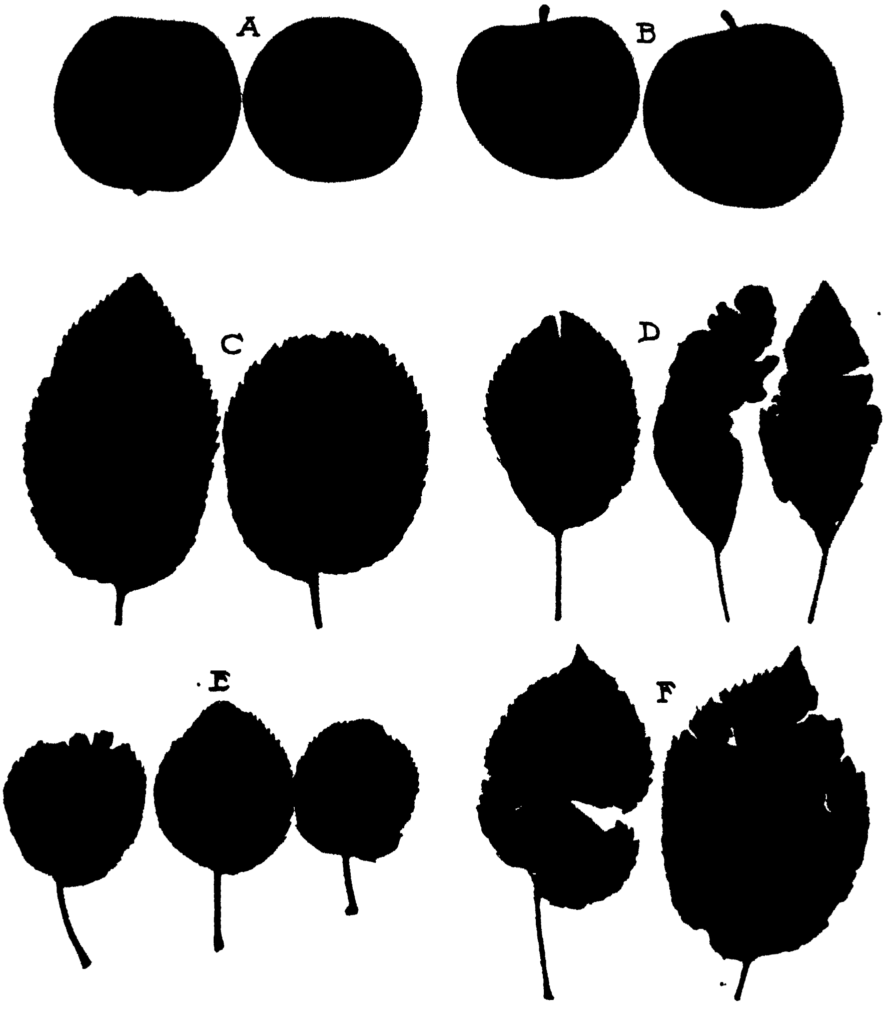

PLATE XIV. Various types of spray injury. A, B, and C.—Scald on fruit and leaves; D.—Edge burn; E.—Yellow leaf; F.—Injury following apple scab.

A CYTOLOGICAL STUDY OF CERATOSTOMELLA FIMBRIATA (E. & H.) ELLIOTT[1]

JOHN A. ELLIOTT

WITH PLATES XV AND XVI

INTRODUCTION

The cytological study here recorded was undertaken as essential in confirming the decision that the so-called pycnidial stage of the sweet potato black-rot fungus is in reality a perfect or sexual stage (4). Due to the unusual nature and behavior of the asci, and to their small size, a study of the nuclear phenomena in the early stages of formation of the perithecia was thought necessary. The presence of sexual organs and nuclear fusion having been established, the study was continued in as much detail as the very minute size of the nuclei would permit. In addition to being of importance in establishing the true nature of the fungus studied, the phenomena observed are likely to be of interest in reference to the nuclear behavoir of Ascomycetes in general, especially as this fungus falls within a group of the Ascomycetes, the Sphaeriales, that has been little studied cytologically. *Gnomonia erythrostroma* was studied by Brooks (3), but its behavior is entirely different from that of *Ceratostomella fimbriata.*

The literature dealing with nuclear phenomena in the Ascomycetes is extensive, covering many different types exhibiting quite varied degrees of sexuality. This literature has been very well reviewed and interpreted by Atkinson (1) and Gwynne-Vaughn (5) from radically different points of view. It is my purpose in this paper to mention only such observations of others as may be directly compared with those made in this study.

METHODS OF PROCEDURE

The material used in this investigation was procured from a culture of the sweet potato black-rot fungus growing on the cut surface of sweet potatoes kept at room temperature in a moist chamber. The fungus developed normally, producing the typical black rot of the sweet potato. In about a month's time heavy fruiting of the supposed pycnidial form of the fungus was noted, and a considerable amount of the material was prepared for making permanent microscopic slides for class use. This work was done by Mr. Sam Poe, student laboratory assistant. The material was fixed in weak Flemming's solution and imbedded in the usual manner. Sections were cut from 3μ to 7μ in thickness, those of $4–5 \mu$ proving the most satisfactory. Flemming's triple stain and Haidenhain's iron-alum haematoxylin without a counter stain gave good results.

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[1] This manuscript was prepared by the late Doctor Elliott. After his death it was sent by Dr. Bradford Knapp, of Arkansas, to Dr. Thomas F. Manns, who, with Dr. W. J. Baerg, of Arkansas, finished certain details and submitted the paper for publication. While it is not as complete and conclusive as it probably would have been had Dr. Elliott lived, it is published with the idea that it may stimulate further work on the problem.—ED.

In the early stages of the formation of the perithecium, the differentiation of the sexual elements, fertilization of the oogonium, and up to the development of the ascogenous hyphae, could be made out nicely from the triple stained slides. During these stages there was little complication in details. The nuclei were relatively large as compared with other elements and the cytoplasm was apparently dilute and did not interfere. Details of the nuclear behavior, from the formation of the ascogenous hyphae to the cutting out of the ascospores, could be followed most readily in sections stained with haematoxylin. This was due to the extreme minuteness of the nuclei in the ascogenous hyphae and the density of the cytoplasm in the developing asci. In spite of their minute size, the nuclei in all their stages stood out sharply and distinctly in the haematoxylin preparations. The nuclei in all stages were too small for the number or behavior of the chromosomes to be observed.

THE DEVELOPMENT OF THE ASCOCARP

Throughout its vegetative stage the sweet potato black-rot fungus is uninucleate. The hyphal cells and both forms of asexual spores clearly showed their uninuclear condition when stained with haematoxylin (Pl. XV, fig. 1). The morphology of these forms, which has been very well shown by Halsted (6) and Halsted and Fairchild (7), needs no amplification here. This uninucleate nature of the vegetative parts simplified the study of the nuclear phenomena of the fungus in the sexual organs and throughout the history of the perithecium, as will appear later.

Whether or not it is essential that the different sexual elements arise from distinct hyphal strands, in several instances two hyphal branches from different sources were observed to form the original hyphal knot from which the perithecium was to develop. Another condition which seems to favor the view of the different origin of the sexual elements is that the perithecia are always formed in groups, usually crowded together in narrow zones and without relation to the abundance of mycelium or of the asexual fruiting stages.

The branch which is to produce the oogonium becomes thickened at the base, the antheridial branch twists itself around this branch (Pl. XV, figs.

2–5) which differentiates into basal cell, oogonium and trichogyne. The nuclei at this stage become prominent and stain very readily, the triple stain being very effective in bringing out the different elements. The trichogyne is continuous with the oogonium and has no nucleus and no dividing wall. The trichogyne apparently fuses at any point it makes contact with the antheridium. A single nucleus from the antheridium passes through the trichogyne into the oogonium. This migration has been observed frequently and in all stages. The nuclei are very small and chances for differences in interpretation are obvious, as a survey of the literature on the subject will show. The simplicity of the sex organs of this fungus, however, makes it seem less likely that misinterpretations would be made here than in the case of some of the much more complicated nuclear phenomena occurring in some of the other oogonia that have been studied. Whatever may be the meaning of the nuclear fusion in the oogonium of *Ceratostomella fimbriata,* I can see no way of avoiding the conclusion that a fusion takes place. Practically all stages in the history of this fusion have been observed, and the oogonium, with a single large nucleus, has been found repeatedly in stages of the development of the perithecium far too late for the migration of the antheridial nucleus not to have occurred. The stages in the fertilization of the oogonium in *C. fimbriata,* except for the presence of the trichogyne, are very similar to those described and figured by Harper for *Sphaerotheca castagnei* (8), *Erysiphe polygoni* (9) and *Phyllactimia Corylea* (10), and by Blackman and Fraser for *Sphaerotheca castagnei* (2). The indications are that in many cases a considerable period elapses between the fusion of the two nuclei in the oogonium and the division of this fusion nucleus into the pairs of nuclei which migrate into the ascogenous hyphae (Pl. XV, figs. 6–11). When the fusion nucleus does divide, the division takes place within the nuclear-plasm (Pl. XV, figs. 12–13), and its first division is not likely to be confused at any stage with the fusion of the male and female nuclei or be interpreted as such, as has been suggested by Atkinson (1). Eight very small nuclei have been observed as the result of this division, but not enough observations have been made to determine whether this is a usual and fixed number.

During the resting stage of the fertilized oogonium the perithecium makes considerable growth. Immediately surrounding the oogonial cell, large thin-walled cells (Pl. XV, figs. 6–13, Pl. XVI, figs. 14–16) originating, apparently, from the unfertilized basal cell of the archicarp, partly fill the cavity of the rapidly growing perithecium. The walls of the perithecium are made up of vegetative cells originating from hyphal branches closely associated with the antheridial and oogonial branches.

Ascogenous hyphae arise from the carpogonium, the nuclei apparently migrating into it in pairs which divide simultaneously throughout the growth of the hyphae. This is a very definite pairing and not, as Gwynne-Vaughn (5) has suggested for some cases, merely an apparent pairing due to rapid successive divisions of the nuclei before they have had time to separate (Pl. XVI, figs. 14–15). The ascogenous hyphae branch throughout the perithecial cavity between the large thin-walled sterile cells, which may occupy considerable of the space against the walls. In some instances the large sterile cells which usually line the cavity fail to develop and the perithecial cavity is entirely empty in its early stages of development until occupied by the ascogenous hyphae. The hyphae may grow around the inner walls of the perithecium or directly across it. Whenever the hyphae come in contact with the large sterile cells, or in the absence of these cells, with the thick-walled cells making up the true perithecial wall, they form a permanent contact with them and become more or less independent of the original base, *i.e.*, the oogonial cell (Pl. XVI, figs. 15–16). When the perithecium has reached the stage of development in which the mother ascus-cells are being differentiated from the hyphal branches, the hyphae appear to be arising from all parts of the inner wall of the perithecium and their primary point of origin is not discernible. It is apparent that the function of the large thin-walled cells is to act as nutrient cells to the ascogenous hyphae. The first strands of the ascogenous hyphae are very fine threads about $\frac{3}{4}$ μ in diameter, but the secondary branches, especially those with secondary attachments, are considerably larger (Pl. XVI, fig. 15). The nuclei are in all cases very minute and may be seen lying side by side even in the narrowest hyphal strands. Asci are first developed at the apex of the perithecium, from which the long beak is simultaneously being produced. The development of asci progresses backward toward the base and outer walls of the perithecium until the walls are reached and the ascogenous hyphae and the nutrient cells are exhausted. The perithecium finally collapses, although vegetative hyphae and the large asexual olive conidia have been observed growing out from the inner walls and partly filling the cavities.

THE DEVELOPMENT OF THE ASCI AND ASCOSPORES

As far as could be observed, the ascogenous hyphae were coenocytic; there did not appear to be even a cutting off, at least in the early stages, of the asci from the hyphae, although this probably occurs in the later stages of development. Nothing in the nature of an ascus hook is produced; the mother ascus-cell first appears as a terminal swelling of a branch of the ascogenous hyphae. It is at first bi-nucleate, which condition maintains until the cell has become about half the diameter of the mature ascus. During the growth of the mother ascus-cell the two nuclei also enlarge greatly (Pl. XVI, figs. 16, 19, 22). The two nuclei fuse to form a single nucleus which is about half the diameter of the fusion nucleus of the fertilized oogonium. This fusion is followed almost immediately by a series of three divisions producing eight nuclei. As in the carpogonium, these divisions occur within the nuclear-plasm, which in many cases is distinctly separate from the cytoplasm of the ascus (Pl. XVI, figs. 16, 23, 25). The free nuclei are finally cut out of the cytoplasmic matrix in the manner first described by Harper (8) and later by many others (Pl. XVI, figs. 16, 23, 27). The presence of a centrosome could not be demonstrated, but the nucleus is drawn out to a point from which the enveloping wall of the spore develops, cutting out the spore. Either before or shortly after the completion of this process the ascus wall disintegrates and soon entirely disappears, leaving the spores (eight or less) more or less closely associated but without an enclosing wall. The spores may continue their development for some time after the dissolution of the ascus, finally attaining a size of 2.5–3.5 by 3–5 µ (Pl. XVI, figs. 28–29). No ascospores were observed in this study which measured more than 5 µ by the longest diameter. Apparently, the measurements given by Halsted and Fairchild (7) are of spores undergoing secondary enlargement, or they are the measurements of the developing asci, which they very closely approximate.

SUMMARY

Several phenomena of interest have been observed in the study of the cytology of *Ceratostomella fimbriata*. 1. There is a fusion of the male and female nuclei in the oogonium followed by a division producing eight nuclei, which migrate into the ascogenous hyphae in pairs. 2. The nuclei of the ascogenous hyphae undergo conjugate division throughout the growth of the hyphae until the ascus mother-cell is produced. 3. The ascus mother-cell is terminal and is not cut off from the hyphae by a wall, so far as has been observed. 4. The ascogenous hyphae fuse with the large uninucleate nutritive cells lining the wall of the perithecium and form new bases from which further development takes place. 5. The ascus disintegrates before the ascospores are entirely mature.

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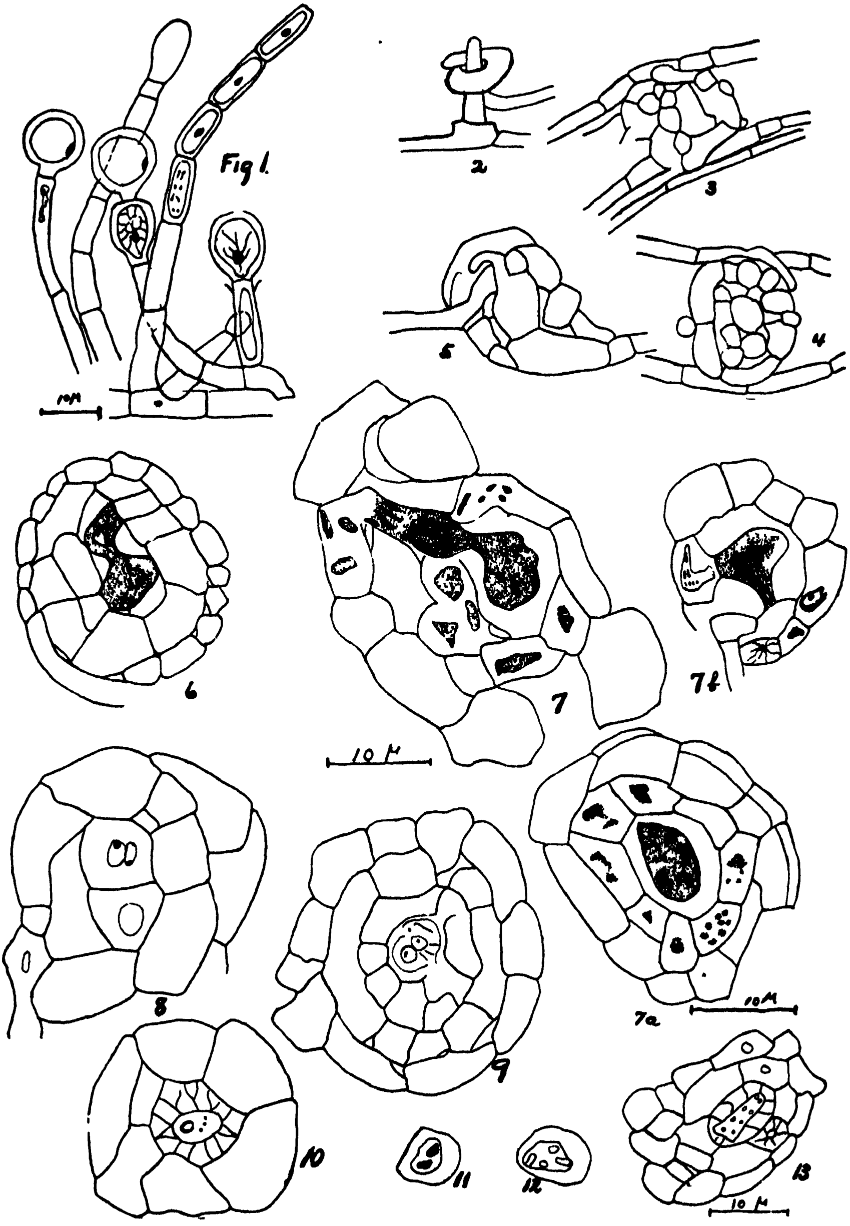

A CYTOLOGICAL STUDY OF CERATOSTOMELLA FIMBRIATA

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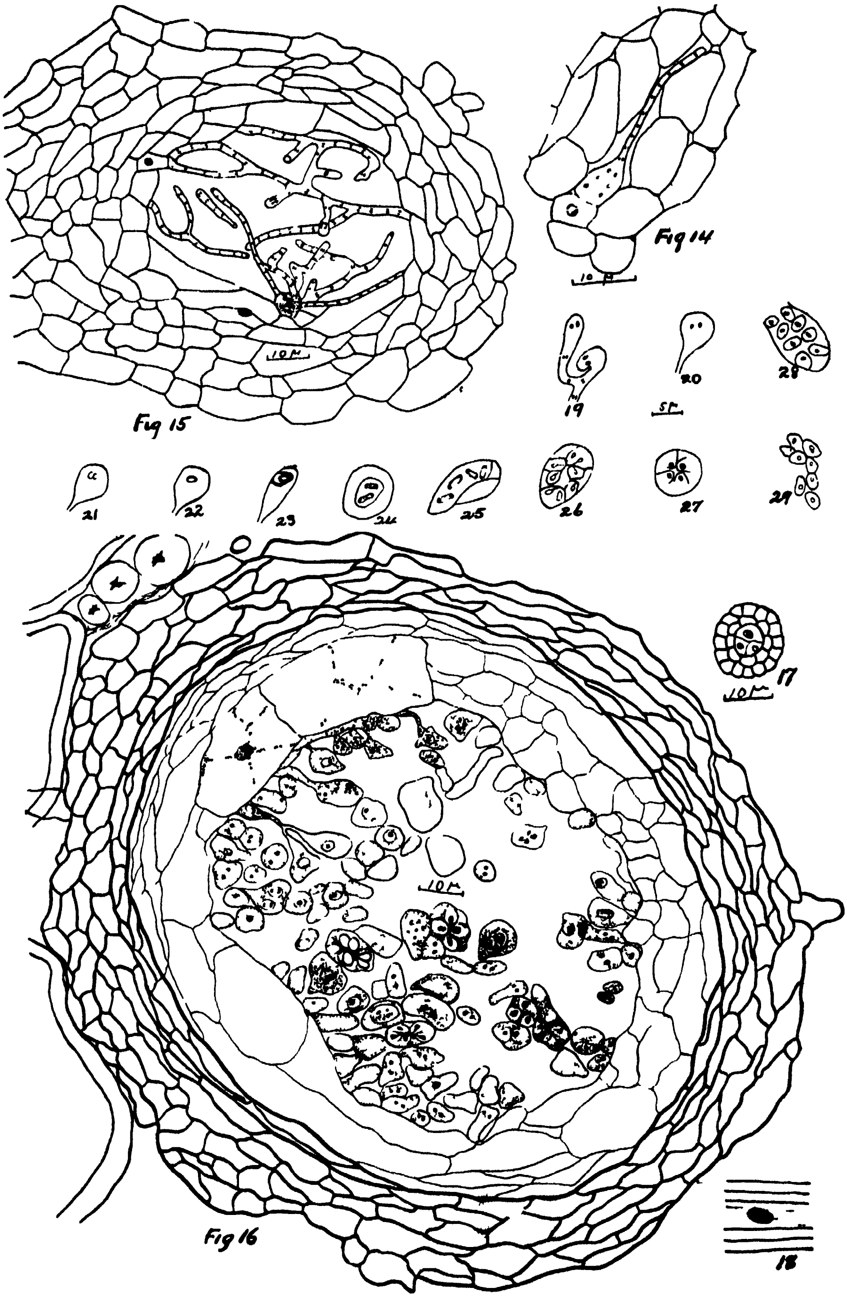

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VARIETAL RESISTANCE OF SWEET POTATOES TO NEMATODES, HETERODERA RADICICOLA (GREAF.) MÜLLER, IN CALIFORNIA

J. L. WEIMER and L. L. HARTER

It is a well-known fact that sweet potatoes are attacked by nematodes[1] and that some injury to the crop results.[2] That this pest is not considered of very great importance on this host probably explains why it has received scant attention as compared with that accorded other sweet-potato troubles. This fact is emphasized by Elliott,[2] who states that the reports by two growers in southern Arkansas of the total loss of their crop due to nematodes were unusual enough to be of interest to other pathologists.

In the spring of 1923 while investigating the diseases of sweet potatoes in California, considerable injury to the slips in the seedbed was observed on a ranch in Los Angeles County. The grower claimed to have lost his entire crop in certain parts of his field the preceding season. Nearby farmers claimed to have suffered considerable reduction to their crops from the same cause. Not only was it apparent that the nematodes were causing more or less direct loss to the sweet-potato crop in the fields, but the presence of large numbers of them in the seed potatoes and on the fibrous roots of the slips showed that sweet potatoes were a big factor in their distribution.

The examination of plants in the seedbeds and in the fields showed that nematodes were quite generally prevalent in sweet potatoes in other parts of the state.

That the nematodes can be distributed with infected seed was shown by the fact that when such seed was bedded in uninfested soil diseased plants resulted.

In one of the fields above referred to it was noted that nematode injury was much worse on some varieties than on others, which suggested the possibility of combating the disease by the use of resistant varieties. Seed potatoes of eight varieties of sweet potatoes (Red Brazil, Red Jersey, Southern Queen, Big Stem Jersey, Yellow Belmont, Nancy Hall, Porto Rico, and Yellow Jersey) were obtained for trial. The seed potatoes were treated in $HgCl_2$ (1:1000) for 10 minutes and then bedded at Garden Grove, Calif., March 5, 1924, in a soil known to be badly infested with nematodes. On

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[1] Bessey, E. A. Root-knot and its control. U. S. Dept. Agr. Bur. Plant Ind. Bul. 217: 1–89. 1911.

[2] Elliott, J. A. Nematode injury to sweet potatoes. Phytopath. 8: 169. 1918.

May 1, when slips were pulled for planting, a few galls were found on the fibrous roots of some of the Red Brazil plants only. That nematodes were present in the soil and that conditions were favorable for infection were denoted by the presence of large galls on the roots of tomato plants set in different places in the bed at bedding time. However, on May 27, when a second pulling of plants was made, numerous galls were found on the roots of both the Nancy Hall and Red Brazil plants. A few very small galls were found on several plants of the Southern Queen and Yellow Belmont varieties. None were found on any of the other varieties. 200, 100, and 50 plants of each variety were planted on infested soil at Garden Grove, Baldwin Park, and Santa Ana, Calif., respectively. These were planted in commercial sweet-potato fields alongside of the commercial crop and given the same care.

Very little difference in the appearance of the plants of the different varieties could be detected, except possibly in the Nancy Hall variety at Garden Grove, in which case the plants were small and lacking in vigor. The plots were dug October 13, 14, and 15, at which time observations were made on the number of galls on the roots of each plant and the yield of each variety. These data are given in tables 1 and 2.

TABLE 1.—*The degree of infection of the different varieties of sweet potatoes grown on the different plots*

Variety	Baldwin Park	Santa Ana	Garden Grove
Porto Rico	Found only about a dozen galls	No galls seen	No galls seen
Big Stem Jersey	No galls seen	A few galls seen	About half a dozen galls seen
Little Stem Jersey	" " "	" " " "	Few nematodes in base of stem; none seen on fibrous roots
Red Jersey	" " "	No galls seen	No galls seen
Southern Queen	" " "	" " "	" " "
Yellow Belmont	" " "	" " "	" " "
Nancy Hall	Numerous galls on fibrous roots. Larger roots rough and irregular. No roughening of potatoes	Numerous galls on fibrous and larger rootlets as well as in the base of the stem	Galls very abundant. Small and large roots deformed and decayed. Root systems very meager. Only a few small potatoes produced
Red Brazil	About same as Nancy Hall	Numerous small galls and swollen rootlets	Numerous galls on fibrous roots. Main stem attacked and considerably roughened

TABLE 2.—*The number of hills, total weight, and weight of marketable potatoes produced; also the average weight per hill of potatoes produced by each variety on the different plots*

	Plot	Varieties							
		Porto Rico	Southern Queen	Red Jersey	Yellow Belmont	Red Brazil	Nancy Hall	Big Stem Jersey	Little Stem Jersey
Number of hills	Baldwin Park	48	47	41	44	47	27	48	39
	Santa Ana	46	75	58	44	44	44	47	39
	Garden Grove	173	189	194	199	160	152	192	190
Total number lbs.	Baldwin Park	75.5	49.5	48	45	44	43.5	53.5	37
	Santa Ana	32	52.5	31	25.5	31	21.5	32	23.5
	Garden Grove	243	236	297.5	182.5	162	77.5	169	183.5
Total number lbs. marketable potatoes	Baldwin Park	66.5	44.5	36	39	32.5	37	32.5	24
	Santa Ana	25.5	37	19	17.5	24.5	12	20	12.5
	Garden Grove	214	199	217.5	141	140.5	59	109.5	111.5
Average No. lbs. marketable potatoes per hill	Baldwin Park	1.39	0.95	0.88	0.89	0.69	1.37	0.68	0.62
	Santa Ana	0.55	0.49	0.33	0.40	0.56	0.27	0.43	0.32
	Garden Grove	1.24	1.05	1.12	0.71	0.88	0.39	0.57	0.59

An examination of table 1 shows that at digging time no nematode galls were seen on any of the plants of the Red Jersey, Southern Queen, or Yellow Belmont varieties. A few galls were found on a few plants of the Little Stem Jersey, Big Stem Jersey, and Porto Rico in some of the plots. On the other hand, the Nancy Hall and Red Brazil varieties were badly infected in all plots. In the case of the two latter varieties the galls were sufficiently numerous to cause a considerable decrease in yield. Owing to the various sources of error in plot experiments of this kind and size, the decrease in yield due to nematodes in these varieties could not be accurately determined from the data at hand. However, the figures in table 2 indicate a considerable reduction in yield of the Nancy Hall variety in the plots at Santa Ana and Garden Grove, especially when compared with the Porto Rico. In the plot at Baldwin Park the Nancy Halls yielded about as well as the Porto Rico; in fact, in this plot, although the plants were fairly heavily infected, the nematodes seemed to have little effect on the yield. A more vigorous vine growth was made in this plot, indicating that conditions for plant growth were such that the infected varieties were able to produce a good crop of potatoes in spite of the nematodes. In the plots at Santa Ana and Garden Grove, on the other hand, poorer conditions for vine growth prevailed, resulting in a correspondingly greater loss due to the ravages of the nematode. This may explain why the Nancy Hall variety grown on infertile, light, sandy soil, heavily infested with nematodes, is frequently a total loss, while when grown on fertile soil, even though nematode-infested, it may produce a fair or even a good crop. However, it is questionable if it is ever advisable to attempt to grow a crop so susceptible to nematodes as either the Nancy Hall or Red Brazil varieties of sweet potatoes on nematode-infested soil. Not only is there the risk of not obtaining a crop, but that of increasing the nematodes in the soil, so that other susceptible crops cannot be grown profitably. The growing of susceptible varieties may also be the means of carrying the nematodes on the plants to uninfested fields. The Red Jersey, Little Stem and Big Stem Jerseys, Porto Rico, Southern Queen, or Yellow Belmont varieties, while not immune, are highly resistant and might be substituted for the more susceptible varieties on nematode-infested land. The more resistant varieties of sweet potatoes can be used to supplant other more susceptible crops on sandy land infested with nematodes.

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POTATO LEAF ROLL AS AFFECTING THE CARBOHYDRATE, WATER, AND NITROGEN CONTENT OF THE HOST

ELMER GRANT CAMPBELL[1]

WITH THREE FIGURES IN THE TEXT

It has been well established by qualitative tests that potato leaf roll is accompanied by an accumulation of starch in the leaves of the host. The earlier literature on this line of work has been reviewed by Murphy (5). Without restating his report it may be sufficient to note that all the references cited by him having a bearing upon the work reported herein have been examined. Approximately all efforts thus far have been not only qualitative in outlook but have pointed solely toward the location of starch.

Esmarch (2), Neger (6), Hiltner (4), and Murphy (5), by the use of the iodine test, have shown that leaf-roll disease is characterized by an accumulation of starch in the leaves. Neger has also shown that diseased leaves are low in water content. Boas (1) found by quantitative methods that the stems of diseased plants were high in amino acids. He found also that in leaf-roll plants there was a higher catalase content—and a higher hydrogen-ion concentration.

The purpose of this short paper is to set forth the results of a brief quantitative study of the carbohydrate, water, and nitrogen contents of leaf-roll potato plants as compared with healthy potato plants.

TABLE 1.—*Dry Weight and C/N Ratio*

Variety	Condition	Percentage dry wt.	Percentage total carbohydrate*	Percentage total nitrogen*	C/N ratio
Rural New Yorker	Healthy	6.79	10.80	5.99	1.80
(Age, 5½ weeks)	Leaf roll	9.49	25.01	4.67	5.35
Irish Cobbler	Healthy	8.94	17.56	3.15	5.57
(Age, 11 weeks)	Leaf roll	10.35	26.75	4.28	6.25
Early Ohio	Healthy	7.17	4.47	5.57	.80
(Age, 11 weeks)	Leaf roll	9.73	16.40	5.50	2.98

* Figured on basis of dry weight.

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[1] The author wishes here to express his indebtedness to Dr. Max W. Gardner and Mr. James B. Kendrick for the plants used in these analyses, to Mr. P. H. Brewer for assistance in laboratory technique, and to Dr. Gardner for helpful suggestions in the preparation of the manuscript.

MATERIALS AND METHODS

Three varieties of potatoes were used in this experiment: Rural New Yorker, Irish Cobbler, and Early Ohio. Healthy and leaf-roll plants were grown under identical greenhouse conditions. Before the time of tuber formation, the plants were clipped at the surface of the soil (healthy and diseased at the same time), weighed, and immediately crushed in 95 per cent alcohol, to which calcium carbonate had been added in amount of one gram per liter. The pulp was placed inside an electric oven and brought to a constant dry weight. It was then ground to a fine powder and used for quantitative determinations as follows: twelve grams for the reducing sugars, two grams for the polysaccharides, and two grams for the total nitrogen. For the determination of the reducing sugars and the non-reducing sugars the official Fehling solution method was used. For the polysaccharides the official hydrochloric acid method of hydrolysis was used, after which the determinations were made by the same method as for the sugars. The total nitrogen was determined by the usual Kjeldahl-Gunning method. All percentages were figured on the basis of dry weight.

TABLE 2.—*Carbohydrates in Percentage of Dry Weight*

Variety	Condition	Reducing sugar	Non-reducing sugar	Polysaccharides
Rural New Yorker	Healthy	0.60	0.40	9.81
(Age, 5½ weeks)	Leaf roll	1.30	1.66	22.05
Irish Cobbler	Healthy	0.68	0.31	16.57
(Age, 11 weeks)	Leaf roll	1.38	1.70	23.67
Early Ohio	Healthy	0.10	0.27	4.10
(Age, 11 weeks)	Leaf roll	1.15	1.80	13.45

RESULTS

Invariably the leaf-roll plants were higher in percentage of dry weight. This fact is shown graphically in figure 1, and numerically in the dry weight column of table 1. The ratio of carbohydrate to nitrogen runs consistently higher in the diseased plants, as is shown in the right-hand column of table 1 and by fig. 3. All forms of carbohydrate, reducing sugars, non-reducing sugars, and polysaccharides, are higher in the diseased plants. The data are given in table 2. The total carbohydrate in healthy as compared to diseased plants is shown graphically in figure 2.

SUMMARY

Let it be emphasized here:

1. That the plants involved in this study were grown under identical greenhouse conditions.

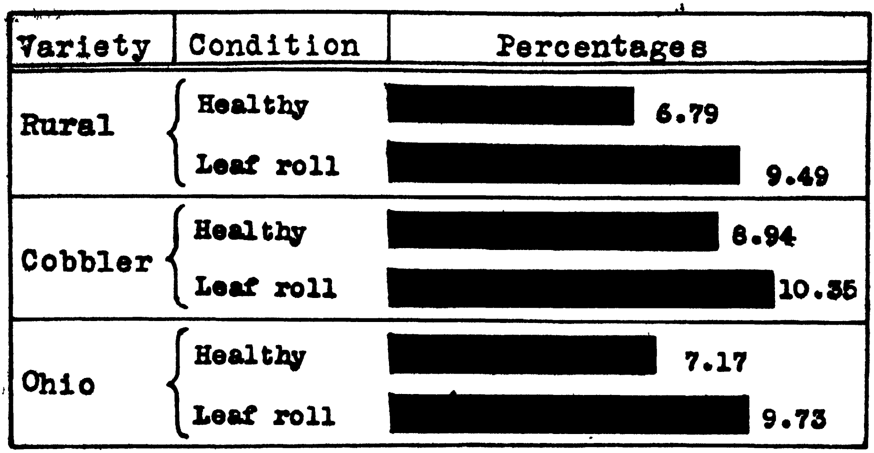

FIG. 1. Dry weight ratio.

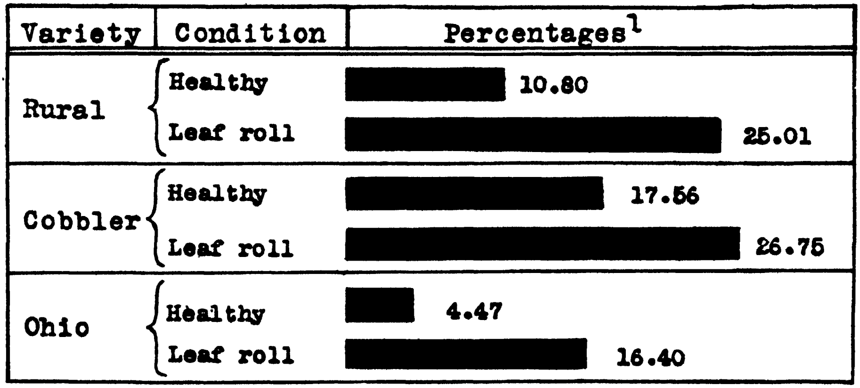

FIG. 2. Carbohydrate content. [1] Figured on basis of dry weight.

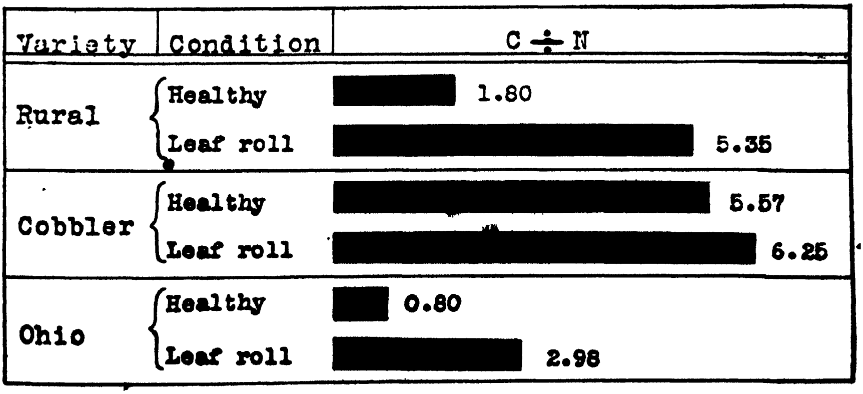

FIG. 3. Carbohydrate-nitrogen ratios.

2. That the plants were harvested prior to tuber formation.
3. That the entire above-ground portion of the plants was crushed and used in the analyses.
4. That in comparison with the healthy, the leaf-roll plants invariably showed: (a) a higher percentage of dry weight; (b) a higher percentage of carbohydrates, sugars as well as starch; (c) a higher C/N ratio; (d) and a percentage of total nitrogen approximately equivalent to that of the healthy plants on the basis of dry weight, and higher on the fresh weight basis.

REMARKS

From these facts it does not seem probable that a retarded translocation rate is the only factor causing a simultaneously high carbohydrate and low water content in the leaf-roll plants. A high sugar content under conditions of abundant external water should normally mean a correspondingly high water content for the plant, but in this instance such is not the case. It may be that the disease virus in leaf-roll plants has retarded the movement of the transpiration stream, or that possibly it has somehow stimulated photosynthetic action temporarily beyond the normal. The operation of either of these assumed possibilities or a combination of them might change the dry weight-carbohydrate ratio to an abnormal figure.

Further studies of these physiological processes are being planned in this laboratory.

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PHYTOPATHOLOGICAL NOTES

Taphrina communis and Taphrina mirabilis. My attention has been called to the fact that Giesenhagen (Die Entwickelungsreihen der parasitischen Exoasceen. Flora **81**: 267–361. 1895) used the combinations *Taphrina communis* and *T. mirabilis* for the species described by Sadebeck and Atkinson, respectively, as *Exoascus communis* and *E. mirabilis.* In my paper in the February issue of Phytopathology (Cultural and morphological studies of some species of *Taphrina*), therefore, the specific names in question should appear as *Taphrina communis* (Sadeb.) Giesenhagen and *T. mirabilis* (Atkinson) Giesenhagen.—ELLA M. MARTIN, Illinois Wesleyan University, Bloomington, Illinois.

BOOK REVIEWS

Laboratory Outlines in Plant Pathology. By H. H. Whetzel, L. R. Hesler, C. J. Gregory, and W. H. Rankin. Second Edition. 231 pages. W. B. Saunders Co. 1925.

The contents of this work bear out the announcement that the second edition of this valuable laboratory guide has been "completely revised and rewritten by the senior author." When Professor Whetzel proposed to turn over to the younger men the chief executive responsibilities of the Cornell Department of Plant Pathology in 1922, he stated that he wished to give part of the time thus freed to book-making. While all American teachers of applied botany are awaiting with especial eagerness the appearance of his promised new "text book," we welcome meanwhile this careful revision of the laboratory outlines. Probably in no other laboratory have so many of these diseases received first hand investigation. Hence the outlines in every chapter may be taken as authoritative guides checked against long experience with classroom use.

The new publishers have given it a clean, attractive make-up, and it bears throughout evidence of careful attention to typographical form and proof-reading. The general plan, as one would of course expect, follows that of the first edition, grouping the examples under the three headings, necrotic, hypoplastic, and hyperplastic diseases. The writer pointed out in his review of the first edition (PHYTOPATH. 8: 6. 1918) that this sequence of laboratory exercises represents the unique experimental contribution of this book in the field of plant pathological teaching. Since most of us school teachers are peculiarly apt to become slaves to usage and easy habit followers, it is well to have a live-wire progressive like Whetzel jar us into a realization of the fact that success in teaching depends little on form or sequence of topic and much on vitalizing energy and contagious enthusiasm. Moreover, as stated in the preface, there are here included practically three times as many exercises as will ordinarily be covered in the usual undergraduate course in plant pathology, hence each teacher must select those exercises best suited to personal use. The mode of treatment is so admirably standardized and the exercises have such uniformity in mode of treatment that it seems to the reviewer possible for each teacher to take them up in almost any order he may wish. Some might criticize these outlines as lacking in organized effort at the comparative or progressive development of the topic which is typical of our better elementary laboratory courses in biology. The experience of the writer would indicate that plant pathology does not impose upon the teacher the same logical "simple to complex" sequence. Consequently, is it one of the many commendable features of these outlines that they present the wholesomely stimulating challenge to the teacher to formulate and develop his own central theme? As the authors wisely state in the Preface, "Although the acquisition of a body of facts is an important and necessary part of such a course, a more vital feature is the training in logical methods of acquiring them." It is evident from the general directions (pp. 11–28) that with the authors these would include training in critical comparison of "symptoms" and "signs" and in the use of literature. The preparation of a bibliography is one of the features where wholesome training in exactness is indicated by the detailed outline (pp. 13–17). If a suggestion were to be added by the reviewer to these already full directions it would deal with two points in which students commonly need guidance and in which the editing of the reference lists of the body of the text is not wholly consistent (*e.g.*, compare the "References" cited, pp. 37, 43, 53, etc.). These concern the questions of whether or not one should or should not capitalize the leading words in cited titles and whether, in such titles, the binomial plant names should or should not be italicized. Unquestionably this training in exactness is favored also by the carefully prepared "glossary." It is to this, probably, that most mature phytopathologists will turn first to seek helpful additions or more refined distinctions for our vocabulary. As supplementing certain useful terms (inoculum, epiphytotic) which the first edition did much to fix in American usage, we find here some interesting additions. Especially one notes "suscept" as differentiated from our familiar "host" and "physiogenic" as applied to "a disease primarily caused by some non-living factor." Both of these certainly point to weaknesses in our technical terminology. Probably their acceptance will be further advanced by their usage as exemplified in the anticipated text-book. Meanwhile, the writer wishes to congratulate and thank the authors for their virile leadership in the educational aspects of our profession.

L. R. JONES

Diseases of Crop-plants in the Lesser Antilles. By William Nowell. Published on behalf of the Imperial Department of Agriculture by the West India Committee, London. 383 pages, 150 figures. 1923.

Mr. Nowell has given to planters in tropical regions a most comprehensive, lucid, and practical guide for the recognition and combatting of crop diseases, and he has given to students of plant pathology everywhere a most stimulating and helpful discussion of fundamental scientific principles.

The first part of the work discusses the nature and causation of disease, parasitism, and host resistance and susceptibility; then follows a condensed general outline of causal agents, a few pages being devoted to each of the principal parasitic groups of fungi, with chapters on pathogenic bacteria, infective viruses, phanerogamic parasites, nematodes, insects in their relation to plant diseases, non-parasitic diseases, and entomogenous fungi. General means of prevention and control are treated under such headings as spraying, dusting, disinfectants, wound treatment, elimination methods, disease resistance, and plant disease legislation.

The greater portion of the book is devoted to specific diseases of tropical crops. The treatment of each major disease gives a clear understanding of the symptoms and nature of the disease, the conditions influencing its development, and the most approved control measures. References to special literature are well selected, but include very few more recent than 1920. This is explained by the fact that the work was written during the author's tenure of the position of Mycologist on the staff of the Imperial Department of Agriculture for the West Indies (1913–1920), and there has been some delay in publication. As Prof. J. Bretland Farmer has well said in the foreword: "Mr. Nowell has embraced the opportunity which lay to his hand and has produced a work which in my judgment constitutes a real landmark of progress in the science of the plant, considered in relation to health and disease."

H. R. FULTON

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PHYTOPATHOLOGY

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AUGUST, 1925

THE SO-CALLED STRANDS AND SECONDARY TUMORS IN THE CROWN GALL DISEASE

MICHAEL LEVINE

WITH PLATES XVII TO XX

So far the so-called strands and secondary tumors in neoplasia of plants have been shown to have no significance other than the possible analogy they may bear to metastases in human cancer. Smith (16) claims that *Bacterium tumefaciens* when inoculated into plant tissues produces types of growths that are analogous to sarcomas, carcinomas, and embryomas in animal and human cancer. He makes a general assertion that, while the organism he and his colleagues discovered would not produce cancer in animals or plants, he believes that animal and human cancer are the results of parasites.

The fact that no type of neoplasia in plants was analogous to carcinoma in animals was pointed out by Levin and Levine (3) and others. The sarcoma-like growth produced by inoculation with *B. tumefaciens* into young meristematic tissues of plants has been shown to be granulomatous in nature and not malignant growth in a great majority of cases, by Levin and Levine (3). That leafy shoots in the crown-gall disease are not analogous to the embryomata in animals was later pointed out by Levine (4, 10). The claim made by Smith (16) that the crown-gall organism is an intracellular parasite is denied by Robinson and Walkden (15) and independently by Riker (13, 14). While these results have come from two different laboratories, it appears, according to Smith (17), that the question is not entirely settled. Evidence recently advanced by Petri (12) shows that the Aleppo pine tumors are related to crown galls and are due to an intracellular organism, *Pseudomonas pini* (Vuill.) Petri. Atypical divisions of cells characteristic of human and animal cancer are not found in the neoplastic diseases of plants (11).

The points that are of interest for further study are those referring to secondary tumors and tumor strands. Smith (16) claims that the crown gall sends out "roots" or "tumor strands" into the normal tissues. These extend for some distance into the healthy tissues. These strands consist of immature, actively vegetating cells. These cells are meristematic in nature and are capable of forming medullary rays, tracheids, and sieve tubes.

In animal cancer, metastases occur by lymphatic permeation and more frequently by emboli of the cancer tissue disseminated through the blood and lymph streams.

The question of lymphatic permeation of animal cancer does not appear to be settled. Handley (2), in cancer of the breast, has shown that there may be extensions of tumor cells through the lymph channels of the deep pectoral fascia to the axillary and subclavicular nodes, then through the chest and abdominal cavities, invading all organs. Handley believes this process of lymphatic permeation is the chief method by which cancer is disseminated.

Ewing (1) points out the fact that Handley's studies were made on material at autopsy and that emboli lodging at various points in the chain of lymphatics may grow in opposite directions until they meet and so fill the lymph channel. Ewing sums up by saying, "In general it appears probable that the rapidly growing epidermoid and globular carcinomas disseminate chiefly by lymphatic embolisms while long growing and recurrent tumors especially on the skin often extend by continuous permeation."

Emboli of crown-gall tissue do not seem to be possible. Permeation of crown-gall tissues by means of "roots" and "tumor strands" have been claimed to exist by Smith (16). Secondary tumors arise from these strands. The so-called secondary tumors of plants, if such occur, arise by a method which is not generally accepted even for secondary growths in animals and human beings.

Robinson and Waldken (15) were able to produce crown galls on *Chrysanthemum frutescens* and *Nicotiana affinis,* which are similar in all respects to the crown galls designated by Smith as secondary galls. These workers contend that these so-called secondary growths result from an inoculation and subsequent growth of a meristematic apex. Another type of growth resulting from inoculations of apices is the smooth gall. These galls appear as swellings on the surface of leaves distant from the point of inoculation. These authors regard these cells as the "true secondaries." They claim that continuous chains of bacteria can be traced from the point of inoculation to the smooth growths. The growths are the result of direct stimulation of the tissue rather than the result of tumor strands extending from the crown-gall tissues at the region of inoculation.

Riker (13), in studying crown-gall formation in the tomato, pea, sunflower, and Paris daisy, holds that "secondary galls" appear only in a small percentage of cases, when the inoculations are made in rapidly growing regions. He contends that the inoculations produced by puncturing the tissue release cell fluids into the intercellular spaces of rapidly elongating tissue. This intercellular fluid bears the bacteria relatively short distances in the embryonic tissue. When the tissue matures, however, a crown gall quite distant from the point of inoculation results. Riker contends that the secondary galls and tumor strands are not really secondary growths. He contends that the galls which appear on plant tissues at some distance from the point of inoculation are stimulated to development by some of the bacteria that were introduced at the original point of inoculation. In general, the results of Riker confirm the observations of Robinson and Walkden.

The question of the formation of secondary tumors in plants is of interest in so far as the claim has been made that crown gall is analogous to animal cancer. I have been studying for the past two years the mechanism of the formation of these so-called tumor strands and secondary growths in a variety of plants. I have been engaged at the same time in studying microscopic sections of various plant tissues after inoculation with *Bacteria tumefaciens* for the purpose of locating the organisms in the crown-gall tissue. I shall report on these results in a later paper. In my study of the so-called strands and secondary tumors I have attempted to answer the following questions:

1. How far apart must inoculations with the crown-gall organism be made to affect the tissue between the point of inoculation?
2. What effect has inoculation with *B. tumefaciens* on water-soaked tissues?
3. What effects have inoculations of *B. tumefaciens* on slit tissues?
4. What results from infecting long perforations in tissues with *B. tumefaciens?*
5. What kinds of plant tissues best produce the so-called strands and secondary tumors in the crown-gall disease?

DISTANCE BETWEEN POINTS OF INOCULATION

The effect of inoculating a stem or petiole with *B. tumefaciens* at a number of points at various distances apart is of interest because of the light it may throw on the mechanism of the formation of crown galls and strands in the tissue between the points of inoculation. It must be mentioned that only young tissues susceptible to the crown-gall disease have been used for these studies. The young shoots of the rambler rose, rubber plant, the tomato, and petioles of *Ricinus* plants were used. The midveins of the young leaves of the rubber plant were also utilized. I am emphasizing the fact that apical portions of the shoots were not used. Parts of the plants that were still green and soft and which were back of the region of elongation were tried exclusively in these experiments. The inoculations with *B. tumefaciens* were made at several points ½ cm. to 3 cm. apart. The parts were labeled carefully and then examined from time to time. The inoculations were made during the first week in July, and the galls were collected and studied in October and November. The inoculated plants were removed from the soil and photographed for purposes of record.

In the rose it was found that when the shoots were inoculated with *B. tumefaciens* at two or more points ½ cm. apart, two or more distinct crown galls developed at first. These became oppressed with subsequent growth and frequently showed fusion of the tissue of the galls. In a majority of the cases the galls retained their individuality until they died (Figs. 1, 2, and 3, Pl. XVII). When the inoculations were made from 1 cm. to 3 cm. apart, independent galls were found at each point of inoculation (Fig. 4, Pl. XVII). Free-hand sections were made of the tissue between these galls, but in no case was a new gall developed either behind the point of inoculation or toward the apical portion of the shoot.

The cortical and epidermal portions of the stem frequently seemed to be thickened, but there was no evidence that any strands occurred here. The nature of the structure of these galls was described by Levin and Levine (3). A great number of these experiments were made with the same result.

Similar results were obtained when the rubber plant was inoculated with *B. tumefaciens*. While the number of rubber plants were relatively small, the results were uniform. I found no instance of development of secondary galls between the points of inoculation. In figures 5 and 6 (Pl. XVII) a leaf is shown that had been inoculated on May 27, 1922, by puncturing the midvein at intervals of ½ cm. with a needle containing *B. tumefaciens*. The photographs were taken on November 1, 1924. In these figures we find that some crown galls developed on the ventral surface, while others formed on the dorsal surface of the leaf. In some instances crown galls developed on both sides of the leaf from the same puncture. All these experiments gave the same results. In no case was a crown gall produced on these leaves other than at the points of inoculation.

A large number of tomato plants in my cultures, used in connection with other studies, showed results similar to those noted in the rose and rubber plant. Where the distance between the points of inoculation was small, the galls seemed to fuse. When the distance between the inoculation was greater, independent crown-gall growths appeared. Hand sections were made in a number of cases of young tomato stems immediately after they were inoculated by the puncture method to determine whether the water-soaked areas induced by the injury in inoculation would fuse. It was found that by carefully puncturing the stems the water-soaked areas were confined to a very small radius around the puncture and did not fuse. However this may be, bacteria of the size of *B. tumefaciens* may still find enough fluid liberated by the injury to enable them to move into the spaces between the punctures. The galls first appeared as independent growths but later fused into one large growth. The fact that the stems used for the experiments were young, and possibly capable of elongation, may explain the fusion of these growths. In inoculations made at greater distances, from 2 to 5 cm. apart, the results were similar to those described for the rose. I have studied the petioles of over two hundred plants of *Ricinus*. The petioles were measured before they were inoculated. Inoculations were made at single points at a distance of from 1 cm. to 3 cm. apart. In petioles over 18 cm. long, separate and distinct crown galls appeared from two to four weeks after the inoculation. I was unable to find a thickening of the tissues between the crown galls or swellings arising from crown galls at points other than those inoculated. Such thickenings and swellings occurred only in such growths as I shall describe below for petioles much smaller and younger.

BACTERIUM TUMEFACIENS IN WATER-SOAKED TISSUES

The effects of inoculating water-soaked tissues for the production of strands and secondary tumors were studied.

Young shoots of the raspberry plant (var. Cuthbert) were water-soaked by gently rolling the parts to be inoculated between the finger and thumb. The length of the shoot so treated measured from 8 to 16 cm. This distance was marked off from the rest of the branch by lines made with India ink. The part of the stem directly in back of the growing region was inoculated, so that little or no elongation occurred in the region studied. The tissues were sufficiently young to respond to the inoculation. The middle of the water-soaked area of the shoot was inoculated by pricking it with a needle dipped into a culture of *B. tumefaciens*. After the inoculation was made, the treated portion of the stem was again rolled gently between the fingers and the thumb to facilitate the movement of the plant juices and the bacteria. More than 24 shoots were so inoculated. On all the raspberry shoots so treated, crown galls appeared only at the point of inoculation. There was no visible evidence of crown-gall tissue in the form of strands of globular masses in any other part of the water-soaked area of the stem. The crown galls were large and often split the stem at the point of inoculation. The pith of the water-soaked region of the stem frequently showed signs of necrosis.

Figure 7 (Pl. XVII) shows a shoot of a raspberry plant, a part of which was water-soaked by gentle pressure and then inoculated in that region with *B. tumefaciens* by a single prick of a needle. The water-soaked portion of the shoot is indicated by two marks made with India ink. A crown gall appeared only at the point of inoculation. Free-hand sections of these stems above and below the crown gall were studied, but no indication of a crown-gall tissue appeared.

Figure 8 (Pl. XVII) shows the development of a crown gall in the water-soaked area of the stem. The new growth split the stem. A scar due to the injury induced by squeezing the shoot appears above the crown gall.

It appears from these experiments that the production of the so-called secondary tumors and strands is not facilitated by water soaking. The bacteria which migrate because of the gentle squeezing of the inoculated stem must require, it appears, an additional factor to bring about the proliferation of the cells in parts distant from the point of inoculation. Injury, accidentally induced in the water-soaked area due to excessive pressure, has not resulted in crown-gall formation other than at the point of inoculation.

THE EFFECT OF B. TUMEFACIENS INTRODUCED INTO SLITS MADE IN YOUNG STEMS

In these experiments I have used rapidly growing, and slowly growing shoots of the geranium and rose, and petioles of *Ricinus*. The apices of the branches were slit with a sterilized knife for a distance of from 10 to 12 cm. In many cases the slit involved the petioles of the youngest leaf. The depth of the cut was approximately to the center of the pith. *B. tumefaciens* was then introduced by means of a sterilized needle covered with bacteria and introduced between the cut surfaces of the stem. When a sufficient quantity of the bacteria was left in the slit, the stem was gently pressed until the bacteria were uniformly spread over the cut surfaces. Forty experiments of this kind were made. The plants were examined daily, and a large number of these were photographed at intervals of from 7 to 10 days. In the rapidly growing shoots it was noticed, shortly after the inoculations were made, that the cut surfaces began to separate; at the same time a proliferation of cells occurred in the cambium region along the surfaces. These growths increased in size and finally fused with each other. After a period of from 5 to 6 weeks the cut surfaces began to lie in the same plane and were entirely covered with many small nodules which fused into a mass of crown-gall tissue.

Figures 9 and 10 (Pl. XVIII) show an early and a late view respectively of a growing stem of a geranium, slit for a distance of 12 cm. and extending into the petiole of a young leaf. These figures represent two of a series of photographs made at intervals of a week. At the end of a month a distinct hyperplasia appeared on the cut surfaces (Fig. 9, Pl. XVIII). At the end of seven weeks, the end of the experiment, the entire surface was covered with numerous intumescences which had fused into a uniform mass of crown-gall tissue on the cut surfaces of the stem and petiole. The petiole died five weeks after the inoculation was made, but remained attached to the stem. It was frequently noticed in these experiments that the terminal portion of the stem continued growing and a crown gall appeared at its apex (Fig. 10, Pl. XVIII). The significance of this is discussed below.

In slowly growing geranium stems the cut surfaces begin to separate slowly. Small intumescences appear at the nodes or at the axils of the leaves. These plants failed to develop crown-gall tissue on the entire inoculated surfaces. Figure 11 (Pl. XVIII) is a photograph of a geranium plant inoculated on February 19, 1924, and photographed for the last time on May 3 of the same year. Three months after inoculation the leaves withered. The crown galls remained isolated bodies at or near the leaf axils.

Young shoots of the rambler rose, slit and inoculated in a similar manner, form crown-gall tissue on the inoculated surfaces. Occasionally, however, globular masses of crown-gall tissue appear on the cut surfaces (Fig. 12, Pl. XVII). Young petioles of *Ricinus* likewise give similar results, although, as in the rose, globular crown galls frequently form on the cut surfaces (Fig. 13, Pl. XVII). These results suggested the possibility of inducing similar masses of crown-gall tissue internally by introducing *B. tumefaciens* into a long channel in the stem of a plant. The internal development of such globular masses of crown-gall tissues would bring about structures similar to those swellings described as secondary tumors in this disease.

LONG PERFORATIONS INOCULATED WITH B. TUMEFACIENS

The effects of introducing *B. tumefaciens* into long perforations made in the stems of various plants were studied. For this experiment twenty tobacco plants, twenty geranium plants, and twelve shoots of a rambler rose were used. A stiff wire, 40 cm. long, was pointed and by means of a rotary motion was thrust through the upper portions of the stem of the plant for a distance of from 10 to 20 cm. This needle was moved up and down in the tissue a number of times to widen the channel thus formed. Without removing the needle from the stem, a culture of *B. tumefaciens* was smeared over the tip and upper part of the needle. The needle was again moved up and down in the channel. After the needle was withdrawn, *B. tumefaciens* was applied to the entire surface of the needle and it was again introduced throughout the length of the perforation. Introducing a suspension of the organism in water was not entirely satisfactory. The fluid ran down on one side of the puncture, a large number of the bacteria was lost, and only a limited portion of the internal surface of the channel was infected.

Two months after the inoculations were made all the plants showed crown galls at the points where the needle perforated the surfaces of the stem. The size of the crown gall appeared to be in proportion to the age of the tissue (6). Larger galls invariably appeared at the upper end of the stem.

Figure 14 (Pl. XVIII) is a photograph of a representative specimen of the tobacco stems used in my cultures. It shows the result of an inoculation made two months previously. At each point of inoculation, "A" and "B," distinct globular crown galls appear. Not infrequently leafy shoots covered these crown galls. No distinct globular mass of crown-gall tissue developed along the channel between the wounds made by the inoculating needle on the surface of the stem.

Longitudinal sections made through the line of inoculation on the stem show a distinct proliferation of cells which almost closes up the channel. Such a section of the stem made through, and extending from the points "A" to "B" in figure 14, is shown in figure 15 (Pl. XVIII). The new internal growth consists of a cylinder of tissue around the track made by the needle. In the center of the cylinder of this newly-formed tissue is a necrotic area. Differentiation of the new tissue occurs, resulting in the development of vascular elements. At the upper end of the section, at "A^1", woody elements may be seen extending into the pith. In the region below "A^1", a distinct cylindrical layer of tissue may be observed. The tissue surrounding this cylinder is apparently normal pith. Microscopic examination was made of pieces of pith through which the inoculating needle had passed. Figure 16 (Pl. XIX) represents a quadrant portion of a cross section of the pith through the region of inoculation. The irregularly-shaped wound filled with necrotic material is seen on the upper left in the figure, and is marked "C." The cross section shows the development of at least two distinct zones around the region of inoculation. Immediately surrounding the wound, small parenchymatous cells appear which have a fine granular cytoplasm and apparently normal nuclei. Here the direction of growth appears to be radial. On the periphery of this zone another layer of cells appears. These are smaller, but form a band of tissue in cross section equal to the first zone. This layer of cells appears to be intercalated between the first zone and the apparently normal pith which follows. Figure 17 (Pl. XIX) shows an enlargement of the boundary portion between the first and second zones. The arched layers of cells are suggestive of pressure.

Control plants through which channels were bored from one to two months previously, but which were not inoculated, show changes slightly different. A cylinder of new cells is formed around the wound. The rate of growth appears to be much slower. No second or middle zone appears. The channel in the control plants remains large, and destroyed cells are visible in it.

A longitudinal section (Fig. 18, Pl. XIX) through the pith in this section of inoculation with *B. tumefaciens* shows more clearly the existence of a rudimentary but aberrant organization of fibrovascular bundles. A layer of cells, similar to that seen in figure 16 (Pl. XIX), is found around the opening made by the inoculating needle. The second or middle zone is not well marked in the longitudinal section; nevertheless, it shows vascular fibers three to four cells in thickness. These strands are made up of elongated cells which have smooth or reticulated walls. Two nuclei are visible in many cells. Figure 19 (Pl. XIX) shows these vessels and the associated parenchyma cells under higher magnification. The nuclei appear as two small bodies in the center of each cell. Figure 20 (Pl. XIX) shows the cells with reticulated walls.

Similar results were obtained in my studies on the geranium. Crown galls appeared at the points where the needle perforated the surface. No tumors of a leafy or globular variety appeared between the points where the needle perforated the surface of the stem. The microscopic studies of longitudinal and cross sections of the geranium stem give results similar to those obtained with the tobacco plant. The course of the inoculating needle is marked by a line of necrotic tissue. The cells here are not as markedly oriented as they appear to be in the tobacco stems similarly treated. Differentiation of the newly formed tissue occurs. A number of cells with reticulated markings are found in the middle zone.

Rose shoots inoculated through a channel made in the pith frequently form a globular crown gall at one end of the channel. The end of the channel occupied by the crown gall is generally the younger portion. The older portion of the stem forms a scar. The crown-gall tissue in the surrounding parts along the channel is similar to that described for the tobacco and geranium.

It appears from these sections of the tobacco, geranium, and rose stems that strands of crown-gall tissue can be produced by such inoculations as I have described above. When the inoculation is along a straight path, the new tissue resulting from the inoculation forms a strand-like body in which some of the elements of a conducting system appear. However, no globular growths of crown-gall tissue ever appeared in my cultures between the points of the injured surfaces. These results suggest that the bacteria, in their migration from the primary point of inoculation, require some other factor before they may sufficiently stimulate the normal cells to form globular masses of crown-gall tissue. One of these factors may be an increased supply of oxygen. Transverse punctures intersecting the longitudinal puncture should be made with a sterile needle to test the effect of an additional injury. I have observed that when two longitudinal intersecting punctures are made in the pith of geranium stem, the growth of the crown-gall tissue appears to be more active at the intersection. A relatively large mass of crown-gall tissue is formed in the stem at this point. Even in these cases no distinct globular mass of crown-gall tissue becomes visible on the surface.

INOCULATION OF YOUNG TISSUES

Crown galls that seem to arise at a point distant from the original seat of inoculation have been described. Smith (16) maintains that these galls are "secondary tumors" and borrows this term from the animal pathologist who uses the term to express the relationship existing between a malignant cancer and the growths which arise from it in other parts of the body by means of embolisms. Robinson and Walkden (15) and Riker (13, 14) hold that these so-called secondary growths in crown galls are due to the migration of bacteria which directly stimulate the cells distant from the points of inoculation.

For two years I have studied the effects of inoculating the first two internodal spaces below the bud of over 200 young *Ricinus* plants. These plants were immature at the time of inoculation, barely a foot above the ground. The bacteria were introduced by a single needle puncture. I have observed in these plants, in a limited number of cases, the development of crown galls on the uninoculated node.

Figure 21 (Pl. XVIII) is a photograph of a *Ricinus* stem that was inoculated in the internodal space below the bud. Shortly after the gall appeared, it was noted that the surface of the node above became slightly enlarged on the side inoculated and directly above the gall. There was no visible evidence of any continuity between these two. Two months later, October 21, 1922, when the photograph was made, a distinct smooth swelling appeared on the node; and the two growths were visibly connected by a thickening which formed a ridge on the stem. A longitudinal section of the stem (Fig. 22, Pl. XVIII) shows a distinct hyperplasia of the wood and cortex between the gall and the node.

Figure 23 (Pl. XVIII) shows an older stem inoculated four months previously with *B. tumefaciens* in the two uppermost internodal spaces. Here a third crown gall on the node is shown between the two inoculated internodal spaces. The development was similar to that described above. This crown gall on the node appeared first like a little smooth swelling. As its development proceeded it burst through the epidermis and formed a crown gall not unlike the preceding galls that appear at the seats of inoculation. Longitudinal sections of this stem (Fig. 24, Pl. XVIII) through these galls show a thickening of the meristem with subsequent development of the wood, cortex, and epidermis. These thickenings of the tissue extend in all directions from the point of inoculation. The greatest growth appears to be parallel with the long axis of the stem and on the side where the inoculation was made.

It appears that after inoculation the growth of the crown-gall tissue proceeds in all directions. It is greatest in the vertical axis, as shown by a comparison of the thickness of the wood and cortex of the inoculated and uninoculated sides of the stem. When the longitudinal growth of the crown-gall tissue reaches the node, it ceases. It seems that the limit of its longitudinal growth is reached simultaneously with the attainment of the vertical maximum growth of the tissues of the internodal space. The new direction of growth seems to change at the node. This is at right angles to the axis of the stem, forming a globular mass of crown-gall tissue. The stimulated cambium grows faster than the stimulated cortical cells, and the crown gall bursts through the epidermis, which is slowest to respond, thus forming a gall with a rough surface. It appears that this is the fate of all the smooth swellings if their development is not interfered with. It may be argued that the bacteria, in their migration from the point of inoculation, find a more suitable type of cell for stimulation in the tissues of the node rather than at the internode. I have found no evidence that these so-called secondary galls are due to the migration of the bacteria from the point of inoculation. There appears to be no adequate explanation for the cessation of the movement of the migratory organism and stimulation of cells beyond the region of the node. I have not found these so-called secondary galls in nodes beyond the one above, bounding the inoculated internode. I have been able to isolate *B. tumefaciens* from the portion of the stem above the gall on a branch of rubber plant (5).

It appears that these so-called secondary galls are not the result of permeation of crown-gall tissue as I shall point out below. The entire meristematic tissue of the inoculated side of the stem is involved. It appears that these hyperplasias are growths directly associated with the development and elongation of the stem and that when the main axis of growth occurs in the vertical direction, the elongation of the hyperplasia in the internode proceeds as rapidly as that of the rest of the stem. No visible evidence of these growths appears at this time. When the cells can no longer grow vertically, division and growth of the cells occur parallel with, and then at right angles to, the long axis of the stem; a smooth swelling occurs along the internode from the region of inoculation to the node. A swelling, with subsequent typical crown-gall formation, results at the node.

I inoculated the internodal spaces of the older portions of the stems of 60 *Ricinus* plants as controls. In no case have I found a crown gall in an uninoculated node. Fifty young tomato stems (var. Ponderosa), measuring a foot in height, were inoculated several inches above the ground. In no case have I found a growth of crown-gall tissue attributable to an inoculation at some distant point.

A more interesting case of these extension growths associated with inoculations with *B. tumefaciens* is seen in young, growing petioles of *Ricinus*. These petioles, as mentioned above, were less than 12 cm. long at the time of inoculation. Figure 25 (Pl. XVIII) represents one of a large number of petioles which was so inoculated. A globular crown gall appeared at "A," the point of inoculation, in about ten days. Shortly after that a series of protuberances appeared in succession. These were formed on the side of the inoculated petiole. Each protuberance first gives the appearance of a small swelling below the surface of the epidermis which appears to be stretched. It then cracks, and the rough crown-gall tissue appears under the surface. A similar condition occurs at the older portion of the petiole at "B," inoculated at the same time as "A." Two small protuberances are noted here, the large one extending visibly from the mass of crown-gall tissue at the point of inoculation.

Many of these petioles were fixed, and microtome sections were made shortly after these smooth swellings developed. Figures 26, 27, and 28 (Pl. XX) represent a series of photographs of the tissue between two such swellings as are shown at "A" in figure 25. No distinct permeating tumor strands are found between these small protuberances. The fibrovascular bundles, the cortex, and, to a slighter degree, the epidermal layer have all increased in thickness by increasing the number of elements in each layer. The tissue consists of elongated crown-gall cells. Figure 29 (Pl. XX) represents, under higher magnification, the pith side of the fibrovascular bundles of the section shown in figure 27. The cells are smaller in diameter but longer. They are more numerous and represent a distinct hyperplasia. Nuclei are present in the cells. Cells with reticulated markings appear in abundance, although spiral vessels are few in number. The side of the petiole opposite to the point of inoculation is normal (Figs. 28 and 30, Pl. XX). The section of the fibrovascular bundles shows normal spiral and reticulated vessels. The cortex and epidermis are also normal.

A close examination of the protuberances or the sub-epidermal swellings (Figs. 26 and 31, Pl. XX) shows that they are made up of a number of parenchymatous cells and fibrovascular bundles. These are surrounded by cortical cells which are covered in turn by one or several layers of epidermal cells. The growth of the cortical and epidermal cells keeps pace for a time with that of the cambium elements. In many of these swellings the rate of growth of the isolated masses of cambium is greater than that of the cortical cells. The latter show signs of the effects of pressure in the region of contact. In figure 31 (Pl. XX) the upper crown gall illustrates this point. The lower crown gall in the figure shows the nodule of woody elements broken through the cortex and epidermis. That none of these nodules of differentiated tissues are strands is clearly shown by serial sections.

Young, growing petioles of *Ricinus,* when inoculated at two or more points ½ cm. apart, develop crown galls at the point of inoculation as described above; and, in addition, the tissue between the points of inoculation becomes visibly swollen. Microscopic examination of this tissue reveals the same type of growth as is shown in series figures 26 to 28 (Pl. XX). A cross section of a petiole between and near one of the points of inoculation is shown in figure 32 (Pl. XVIII). The swelling is due to an increase in the cortex and fibrovascular elements. The pith cells also show considerable proliferation. In the region of the pith and near the fibrovascular bundles, a number of isolated tracheids appear. These are shown in figure 33 (Pl. XVIII). These are not tumor strands but form a part of the new growth and result from a differentiation of the tissue.

Very young tissue inoculated with the crown-gall organism produces a hyperplasia which appears to grow not only at the point of inoculation, but also parallel with the direction of the developing organ. This must not be considered as an invasive growth. Only part of the crown-gall tissues forms a globular mass of cells. The rest grows parallel and keeps pace with the growth of the normal parts of the organ. In these petioles, as in the stems noted above, growth of the crown-gall tissue occurs simultaneously with the growth and elongation of the organ and not subsequent to it. It appears from the study of these petioles that the structures that have been referred to as strands and secondary tumors are elongated growths of the crown-gall tissue. These extension-growths exceed the normal growth of the organ, in which case protuberances or sub-epidermal swellings and ultimately crown galls appear at a point distant from the region of inoculation. These growths are parts of the crown gall at the initial point of inoculation and develop simultaneously with it. In this they differ from the permeation metastases in animal and human cancer. These swellings or protuberances of crown galls at points distant from the region of inoculation do not form after inoculation of older petioles and stems.

BUD INOCULATIONS

I have also inoculated terminal buds of *Ricinus* for the purpose of studying the crown galls on the leaves after the buds develop. In a large majority of cases the inoculation results in the formation of a globular, crown gall at the bud region with subsequent dwarfing of the plant (Fig. 34, Pl. XVIII). In a few cases of inoculated buds I have been able to secure overgrowths on the petioles of the leaves of *Ricinus* plants when the buds had developed. Figure 35 (Pl. XVIII) shows the apical portion of a *Ricinus* plant. The terminal bud was inoculated July 28, 1924, the photograph made October 13, 1924. The bud had opened by this time, but only one petiole was infected with crown gall. The new terminal bud and node were transformed and axilliary buds began to grow (see 9, 10). These figures confirm the observations of Robinson and Walkden (15) and Riker (13) for the Paris daisy and the tomato respectively.

I have made a large number of cross sections of the petiole shown in figure 35 (Pl. XVIII) between the crown gall at the blade and the base of the petiole. There is no indication of any strands of crown-gall tissue between the gall at the base of the petiole and the crown gall at the blade end.

Figure 36 (Pl. XX) represents one of a large number of sections made of the petiole shown at "A" in figure 35 (Pl. XVIII). In all these sections the cortex and fibrovascular bundles were examined, carefully for the presence of crown-gall tissue. Sections in the crown-gall region made at "B" (Fig. 35, Pl. XVIII) are shown in figure 37 (Pl. XX). A distinct increase in the number of fibrovascular elements and an aberrant arrangement of these parts are shown. There is no continuity of crown-gall tissue between the crown gall on the stems and the growth of the petiole at "B."

SUMMARY

1. The so-called secondary tumors and strands in the crown-gall disease are formed only in very young stems and petioles.

2. The so-called secondary tumors and strands result from the growth and elongation of the immature tissues. The young infected tissues keep

pace for a time with the elongation and development of the inoculated organ.

3. The so-called secondary tumors and strands begin to form simultaneously with the globular crown galls at the seat of the inoculation and depend upon the development of the organ for their elongation. Permeation metastases in human cancer occur through lymph channels of mature structures (2).

CANCER RESEARCH LABORATORY,
MONTEFIORE HOSPITAL, NEW YORK.

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DESCRIPTION OF PLATES

The photomicrographs were made with the aid of the Bausch & Lomb D. L. type camera. Zeiss objectives and compensating oculars were used as follows: For figure 20—obj. 8, oc. 8, bellows 20 cm.; for figures 17, 19, 29, 30, 33—obj. 16, oc. 8; for figures 16, 18, 26, 27, 28, 31, 32, 36, 37—obj. A_2, oc. 1, bellows 26 cm.

PLATE XVII

FIGURES 1–2. Shoot of rambler rose inoculated with *B. tumefaciens* in back of the region of elongation at two or more points ½ cm. apart, showing a confluence of galls.

FIGURE 3. Longitudinal section of galls shown in figure 2.

FIGURE 4. Crown galls on a young shoot of rambler rose resulting from two inoculations made with *B. tumefaciens* 3 cm. apart.

FIGURES 5–6. Ventral and dorsal view of a leaf of the rubber plant inoculated on May 27, 1922, at a number of points ½ cm. apart. Photographs made on November 1, 1924, show no swellings between the points of inoculation.

FIGURES 7–8. Crown galls on shoots of raspberry (var. Cuthbert) inoculated with *B. tumefaciens* in water-soaked area. Stems between arrows were water-soaked by gentle pressure.

FIGURE 12. Globular crown galls in the inoculated slit surfaces of a shoot of the rambler rose.

FIGURE 13. Globular crown galls on the inoculated slit surfaces of a petiole of *Ricinus*.

PLATE XVIII

FIGURE 9. A photograph showing an early effect of inoculating *B. tumefaciens* into a slit made in the growing portion of a geranium plant.

FIGURE 10. Same plant as shown in figure 9 a month later.

FIGURE 11. Slow-growing geranium with slit apical region inoculated with *B. tumefaciens*, showing crown galls at leaf axils 75 days after inoculation.

FIGURE 14. Tobacco stem perforated and inoculated with *B. tumefaciens*. The distance between "A" and "B" shows the length of the perforation.

FIGURE 15. Longitudinal section of a tobacco stem showing the internal changes in the pith resulting from the perforation and the inoculation with *B. tumefaciens*.

FIGURE 21. *Ricinus* stem inoculated in the internodal space below the terminal bud showing swelling at the node a month later.

FIGURE 22. Longitudinal section through the stem; the swelling is indicated by the arrow.

FIGURE 23. A crown gall on the node resulting from inoculation with *B. tumefaciens* into the young internodal spaces below and above it. The photograph was taken approximately four months after inoculation.

FIGURE 24. Longitudinal section of the stem shown in figure 23.

FIGURE 25. Crown galls on the petiole of *Ricinus* inoculated with *B. tumefaciens* at the points "A" and "B" with small protuberances developing from them.

FIGURE 34. *Ricinus* plant inoculated with *B. tumefaciens* in the terminal bud.

FIGURE 35. Crown gall on the distal end of the petiole resulting from an inoculation of the terminal bud with *B. tumefaciens*.

PLATE XIX

FIGURE 16. Microscopic view of a cross section through the pith of a tobacco stem shown in figure 14 (Pl. XVIII); "C" indicates region of inoculation.

FIGURE 17. Same as figure 16, showing part between first and second zone under higher magnification (see text).

FIGURE 18. Longitudinal section of the pith shown in figure 14 (Pl. XVIII).

FIGURE 19. Longitudinal section showing differentiation of the middle zone (see text).

FIGURE 20. Longitudinal section showing the reticulate cells under higher magnification. (Same as fig. 19.)

FIGURE 32. Microscopic view of cross section of a very young *Ricinus* petiole between two points of inoculation.

FIGURE 33. Same as figure 32,, showing cross section of tracheids in pith under higher magnification.

PLATE XX

FIGURES 26, 27 & 28. A series of sections between two protuberances shown in figure 25 (Pl. XVIII) at "A."

FIGURE 29. A part of the fibrovascular bundles lying near the pith on the crown-gall side of the petiole shown in figure 27.

FIGURE 30. The normal fibrovascular bundles of the petiole on the side opposite to the crown gall shown in figure 27.

FIGURE 31. Longitudinal section of a crown gall on a *Ricinus* petiole in which the woody nodule has broken through the thickened cortex.

FIGURE 36. Cross section of a petiole shown in figure 35 (Pl. XVIII) with normal arrangement of the fibrovascular bundles.

FIGURE 37. Cross section of the same petiole near the crown gall.

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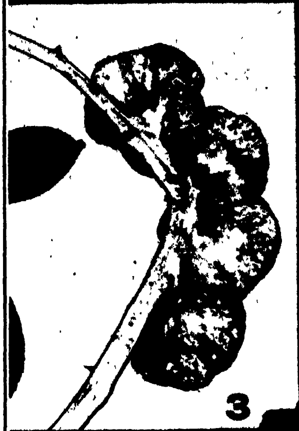
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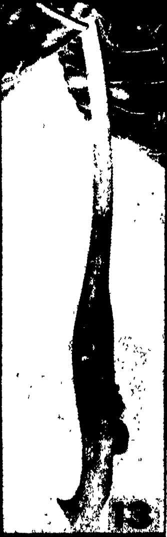
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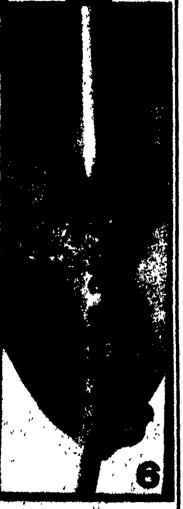
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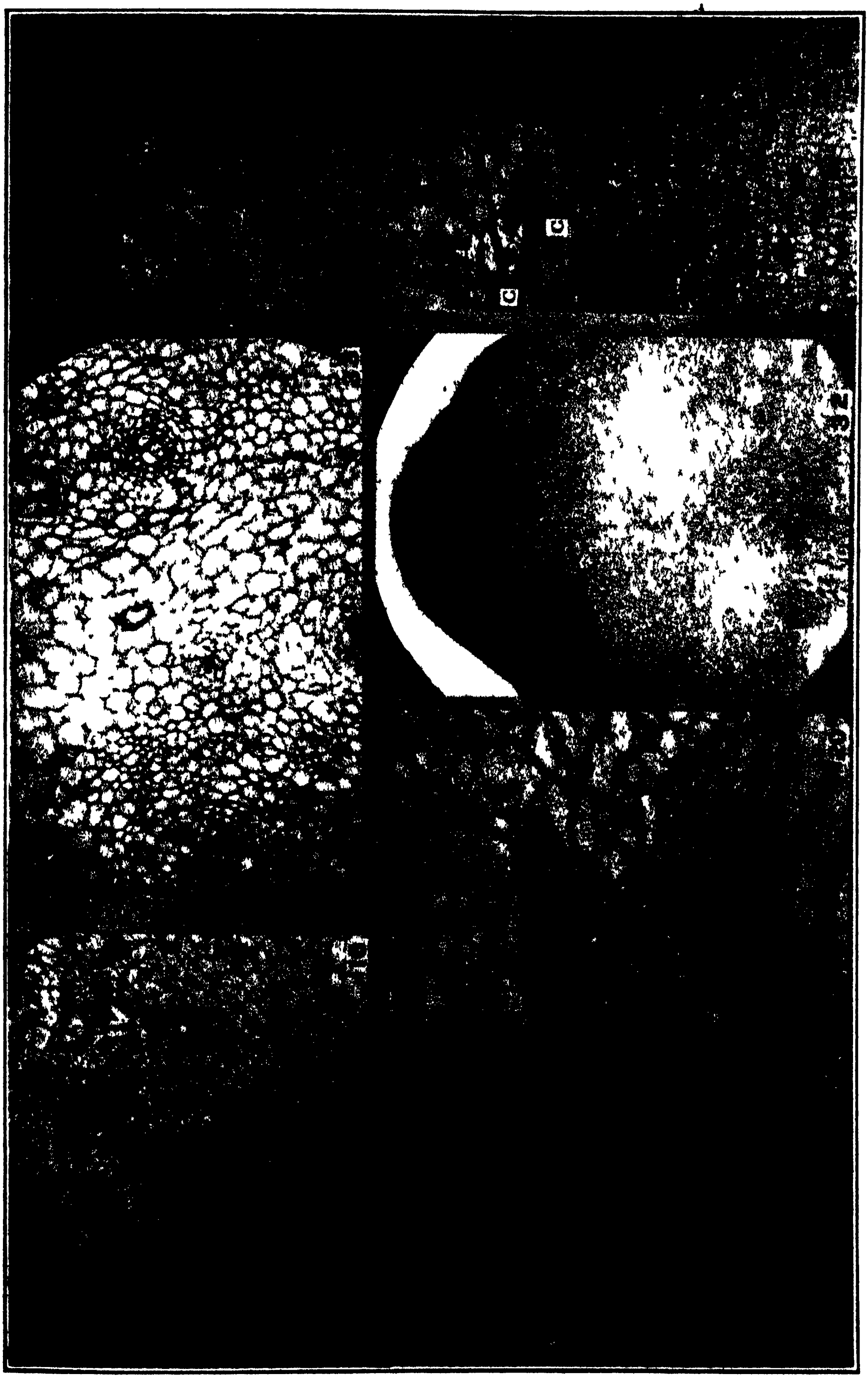
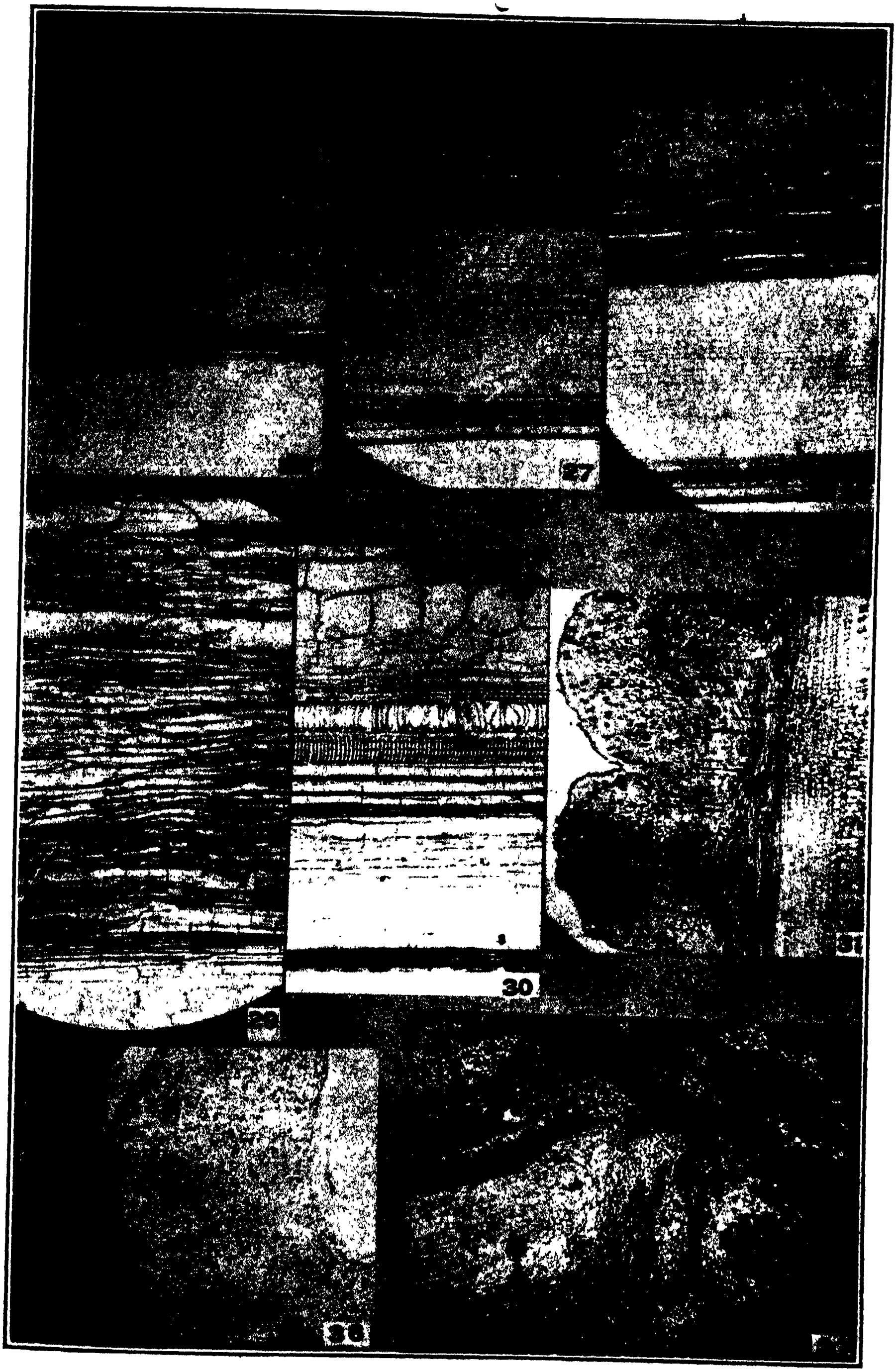
PLATE XVIII

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CLADOSPORIUM SPOT OF COWPEA[1]

MAX W. GARDNER[2]

WITH PLATES XXI TO XXIII

In variety plots of cowpeas (*Vigna sinensis* Endl.) grown at Lafayette, Indiana, a peculiar spotting of the pods of the Early Buff variety was observed in August, 1923, and again in August, 1924. This spotting was in the nature of irregular, black, scabby lesions, distinctly different from the smooth or sunken, circular, maroon lesions of the bacterial spot disease[3] which was being investigated at that time. The trouble was confined to the Early Buff variety, and a more careful inspection further revealed that the peduncles and to a less degree the growing tips of the stems of this variety were thickly beset with small, sunken, purplish lesions which were likewise not to be found on any other variety in the plots. Microscopic, cultural, and inoculation tests proved that this disease was due to an apparently undescribed species of *Cladosporium,* herein designated as *Cladosporium vignae* n. sp., which evinces a predilection for very young, rapidly growing tissues and which has been found to be a seed-borne parasite.

SYMPTOMS

The lesions on the pods were conspicuous, dark purple or black, roughened or scabby spots, very irregular in shape and usually from 2 to 6 mm. in diameter, sometimes larger (Pl. XXI, figs. A to D). The margin of the lesion was irregularly lobed, slightly elevated, and dark purple in color, while the center of the lesion was slightly sunken, brown, tan, or grayish in color (Pl. XXI, fig. D) and sometimes covered with a velvety greenish layer, the spores and sporophores of the causal fungus. In older lesions the central tissue was corky and cracked (Pl. XXII, fig. F) and tended to scale or slough off. Late infection was in the form of numerous very small, black specks (Pl. XXI, fig. A). When the pods matured and became light colored, the lesions were much more conspicuous. This disease differs from bacterial spot in that the pod lesions were often scabby and were darker colored, more conspicuous, and, as a rule, more numerous.

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[1] Contribution from the Botanical Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

[2] The writer is indebted to Prof. H. S. Jackson or advice and to Mr. James B. Kendrick for assistance. Dr. W. J. Morse, of the U. S. Bureau of Plant Industry, very kindly supplied seed of cowpea varieties.

[3] Gardner, Max W. and James B. Kendrick. Bacterial spot of cowpea and lima bean. Jour. Agr. Res. (In press.)

Owing to the fact that infection can occur, apparently, only when the pod is very young, the lesions usually interfered to some extent with the normal growth of the pod and hence caused considerable constriction and bending of the pods, the concavities being occupied by lesions (Pl. XXI, fig. A; Pl. XXII, figs. C and E). Heavy infection of very young pods sometimes resulted in their death. One case of a shrivelled dead ovary, 5 cm. in length, bearing dense, olive green, velvety masses of the spores and sporophores of the fungus was observed.

A large number of infected pods were opened and in a few instances the lesions were found penetrating through the pod wall into the seed. In one of these cases the fungus was found sporulating at the center of a reddish brown spot on the seed coat. In another case in which the lesion was on the ventral suture of the pod, to which the seeds are attached, the underlying seed was much stunted, and the fungus was found sporulating on the membranous inner lining of the pod. Other fungi, such as *Alternaria*, were often present in the old pod lesions.

In the field in the late summer and fall, infection of the peduncles was particularly abundant and conspicuous, and consisted of numerous elliptical, sharply and deeply sunken, dark purplish spots, thickly clustered toward the apical end of the peduncle (Pl. XXII, figs. A and B). These spots varied from 1 to 6 mm. in length, the larger ones being more distant from the apex, more scattered, and usually showing a tan center on which the greenish sporulation of the fungus was frequently visible. Older lesions were sometimes reddish brown or tan and were usually drawn out at either end into rather sharp points (lens-shaped). Occasionally peduncles were killed outright by the coalescence of the lesions.

Infection near the growing tip of the stem very much resembled the peduncle infection except that the lesions were smaller, less deeply sunken, and less numerous (Pl. XXII, fig. D). The lesions were dark purple at first and later developed a tan center, and were usually 1–3 mm. in length. Similar small, dark purple lesions occurred rather abundantly on the petioles, petiolules, and main veins of the leaves (Pl. XXII, fig. D). The lesions were often on the veins on the lower side of the leaf and by inhibiting growth were very likely to cause considerable distortion of the leaf. On the leaf blade very small, circular, sunken, dark purple- or maroon-bordered and tan-centered spots, 0.5 to 1 mm. in diameter, were formed (Pl. XXIII, fig. A). These were usually surrounded by a yellowish translucent margin. Similar lesions were produced on the stipules, bracts, and calyx. Under field conditions it may be said that in general the *Cladosporium* lesions were smaller, darker, and more sharply defined than bacterial spot lesions.

The first leaves of inoculated seedlings in the greenhouse were considerably distorted and curled upward by the numerous lesions. The latter were from 1 to 3 mm. in diameter, faded green or grayish, and were soon covered on both sides with the dusty spores of the fungus. Such leaves were soon killed and seedlings were sometimes killed outright by heavy infection. In cases of less destructive infection the leaf lesions were maroon- or purple-bordered, brown or tan spots, irregular in outline, sunken at the center, and about 2 mm. in diameter (Pl. XXIII, fig. D). Much crinkling and tearing of the leaves was produced (Pl. XXIII, fig. C).

Sharply sunken, lens-shaped, grayish or yellowish lesions, 2 to 4 mm. in length, developed in abundance on the hypocotyls and epicotyls of inoculated seedlings (Pl. XXIII, figs E, F, and G). At the center of the lesion the olive-green dusty layer of spores was produced. The lesions near the tip of the epicotyl became much larger, and in the case of seed-borne infection hypocotyl lesions up to 20 mm. in length were produced, usually emanating from the cotyledon scar and often penetrating half-way through the hypocotyl. Longitudinal fissures often accompanied these lesions. In older plants the lesions on the peduncles 5 days after inoculation were sharply sunken, elliptical, water-soaked spots with a faint brownish margin and about 1 mm. in length.

CAUSAL ORGANISM

Reference has been made to the presence of a *Cladosporium* sporulating at the centers of certain lesions. Poured plate isolations from the crushed tissue of pod lesions proved that these were not caused by *Bact. vignae.* Poured plate isolations from the fungous growth on the surface of pod, peduncle and stem lesions yielded similar *Cladosporium* colonies, as did also plates poured from the fungus on the small blighted pod 5 cm. in length, previously mentioned. Tissue plantings yielded the same fungus.

Portions of host tissue bearing lesions were cut out, immersed in alcohol and then in a 1 to 1,000 solution of mercuric chloride 1–2 minutes, rinsed in sterile water, and planted in poured plates of potato agar. What appeared to be the same *Cladosporium* developed on 6 of the 15 pod lesions, 9 of the 31 leaf-blade lesions, 3 of the 7 petiole lesions, 3 of the 18 stem lesions, and 12 of the 20 peduncle lesions thus tested. Usually the fungus sporulated directly on the upper surface of the tissue fragment. The same fungus was similarly isolated from a seed from an infected pod. By successful atomizer inoculation of seedlings with a spore suspension, the pathogenicity of the fungus was proved for cultures isolated by the poured-plate method from pod, peduncle, and stem lesions.

The colonies in plates of potato dextrose agar poured from a spore suspension were from 5 to 8 mm. in diameter in 3 days at room temperature, and the surface of each colony was almost completely covered with spores, a circumstance which illustrates how promptly sporulation occurs. The mycelium remains prostrate and becomes olivaceous black, a fact to which no doubt the dark color of the host lesions is due (Pl. XXI, fig. E). The dusty sporulating surface approximates first the vetiver green, then the Lincoln green, and finally the deep grayish green of Ridgway's Color Standards. Excellent growth occurs on other media such as oat and lima bean agar.

Spore germination occurred in exposed water drops at room temperature, and the germ tubes were 1 to 4 times the spore length in 18 hours. In similar drops to which fragments of a cowpea leaflet were added, a higher percentage of the spores germinated, and the tubes were from 4 to 8 times the spore length in 18 hours. However, success was not always obtained in germination tests. Vigorous germination occurred in infection drops on the leaves of inoculated seedlings. In one trial in which vigorous germination occurred in a drop of tap water on a slide, many of the spores which had remained floating had at the end of 48 hours sent long germ tubes down into the liquid and branched chains of small spores up into the air. This illustrates again how promptly the fungus proceeds to spore production.

MORPHOLOGY AND TAXONOMY

The spores are produced in a cluster of branched chains at the end of an unbranched sporophore. In agar media there is no aggregation of hyphae preliminary to the production of sporophores, but the isolated hyphae emerge directly from the medium and produce an apical cluster of branched spore chains. Ten or more ultimate chains may be formed, each consisting usually of from 2 to 5 spores which are much smaller than the basal spores at the center of the cluster. The spores are light brown in color and are produced acrogenously, that is, the apical spore of each chain is the youngest. The spores produced on seedlings 5 days after inoculation varied in length from 7 to 22 μ while the width varied from 3 to 5 μ. In agar culture spores as short as 6 μ are formed. The spores are somewhat pointed at the ends and the smaller spores are lemon-shaped while the larger ones are cigar-shaped. On each end of the larger spores is a small, thickened disk, apparently the point of attachment to the sporophore or adjacent spores. The spores are usually one-celled, although the larger spores are sometimes two-celled. The spores are easily detached and carried by air currents as was indicated by the heavy seeding of new colonies obtained by blowing the breath across a plate colony. In germination the spores become swollen and may become darker brown and septate.

The sporophores from seedling lesions 5 days after inoculation varied from 40 to 80 µ in length and were darker brown and thicker walled than the spores. The sporophores are unbranched and contain one or two cross-walls and frequently a bend or crook where the sporophore has apparently continued its growth after producing a spore or spore chain. Spore production may begin before the sporophores are full grown. Cross sections of the lesions show that the sporophores are numerous and may be solitary or two or three in a group.

No record of any *Cladosporium* parasitic on cowpeas has been found, and the fungus under consideration differs from the few species described on legumes. The spores of *Cladosporium pisi* Cug. and Macch.[4] on *Pisum* are recorded as smaller with the smaller ones usually 2-celled. Attempts to infect field pea seedlings with the cowpea fungus have been unsuccessful. The spores of *Cladosporium infuscans* Thum.[5] on *Desmodium* are described as usually septate. *Cladosporium album* Dowson[6] described on *Lathyrus* differs in that it is white. Apparently the cowpea parasite is an undescribed species and for it the binomial, *Cladosporium vignae* n. sp., is suggested. It is described as follows:

Cladosporium vignae n. sp.

Sporophores solitary or in groups of two or three, unbranched, often with a bend or crook, septate, brown, 40 to 80 µ in length. Spores borne in an apical cluster of branched chains, acrogenous, light brown, ovoid to ellipsoidal, somewhat pointed at the ends, 7 to 22 µ by 3 to 5 µ, usually 1-celled, the larger spores sometimes 2-celled. In culture the submerged mycelium is olivaceous black, the dusty spore layer, vetiver, Lincoln, or deep grayish green. Parasitic on cowpea (*Vigna sinensis* Endl.), especially the Early Buff variety, producing black scabby lesions on the pods, purplish sunken lesions on the peduncles, stems, and petioles, and very small dark spots on the leaves. Also parasitic on *Vigna sesquipedalis* Wight. Only young growing tissues are susceptible. The fungus is seed-borne and seedling infection occurs. Type locality: LaFayette, Ind. Specimens deposited in the Pathological Collections, U. S. Bureau of Plant Industry, Washington, D. C.

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[4] Cugini, G., and L. Macchiati. Notizie intorno agli insetti, acari & parassiti vegetali osservati nelle piante coltivate & spontanee del Modenese nell' anno 1890. Boll. d. R. Staz. Agraria di Modena 10: 89–107. 1891.

[5] Saccardo, P. A. Sylloge fungorum 4: 361. 188‹.

[6] Dowson, W. J. Contributions from the Wisley laboratory. XLIV. A new disease of sweet peas. Jour. Roy. Hort. Soc. 49: 211–221. 1924.

PATHOGENICITY

Atomizer inoculation of Early Buff cowpea seedlings in the greenhouse with a suspension of the spores resulted in abundant infection of the young rapidly growing organs. Nine seedlings one week old which were inoculated and placed in a moist chamber on October 11 showed numerous lesions on the young first leaves, epicotyls, and hypocotyls within 48 hours. These lesions increased in size to such an extent that at the end of one week 6 of the seedlings were dying. Abundant sporulation occurred on the lesions. No infection occurred on 5 seedlings sprayed with water and incubated as controls. The fungus was reisolated by plates poured from a suspension of the spores produced on an epicotyl lesion, and with this culture infection was obtained in later inoculations.

Ten New Era and 6 Whippoorwill cowpea seedlings one week old were similarly inoculated on October 11. Numerous very small, black, sunken, shiny spots, 0.5 mm. in diameter, appeared on the first leaves of both varieties (Pl. XXIII, fig. B) and a few small elliptical lesions from 0.5 to 1 mm. in length developed on the epicotyls and hypocotyls just above and below the cotyledon scars. Necrosis and blackening occurred much more promptly in the lesions on these varieties than on the Early Buff, but the lesions remained much smaller and showed little or no sporulation of the fungus. New Era and Whippoorwill seedlings sprayed with water as controls remained free from infection. These tests showed that these two varieties were not immune but were not nearly as susceptible as the Early Buff variety.

To illustrate how soon the tissues become resistant to this fungus, inoculation tests made one week later with seedlings of the three varieties planted at the same time as those used in the tests described above, hence 2 weeks old, resulted in no infection. No new leaves had developed on these seedlings and the first leaves, epicotyls, and hypocotyls had evidently become somewhat resistant. Nine and ten days later, inoculations on similar seedlings, now over three weeks old, and some with the first compound leaves unfolding, resulted in abundant infection of the unfolding leaves of certain of the Early Buff and New Era seedlings (Pl. XXIII, fig. C). Petioles, stipules, and bracts were also infected. With one or two exceptions no infection occurred on the stems or first leaves except on the ventral or upper surface of the very short petiole of the first leaf, a region which apparently remains susceptible. Lesions on this area were purplish and lace-like, and from two of these the fungus was cultured. In one case a few lesions developed on the stem near the tip and in another case on the stipule of an older leaf.

In later inoculations of seedlings of which the second compound leaf was unfolding, infection occurred only on the latter and not on the older leaves, so that one is led to conclude that, in general, only the young growing organs are susceptible, a condition which field observation likewise suggests. Under favorable conditions, however, infection of such organs occurs with great rapidity and virulence, and visible lesions already causing crinkling of the leaves may be present within 48 hours after inoculation. Inoculation of older plants resulted in numerous lesions on the very young leaflets, stem tips, peduncles, and bracts, and in 5 days the fungus was sporulating profusely on the bracts surrounding the immature floral organs.

In order to determine varietal susceptibility and host range, greenhouse inoculation tests were made upon seedlings of 15 varieties of cowpeas and two related species with the result that the Early Buff variety proved most susceptible while the Progressive White variety ranked rather close to the former in its susceptibility. Next to these were the Columbia variety and the related species, asparagus bean (*Vigna sesquipedalis* Wight). Then followed a group of less susceptible varieties consisting of Groit, Whippoorwill, Large Blackeye, Early Red, Victor, New Era, Red Ripper, Clay, Iron, and Brabham. The Taylor and Early Black varieties and the species catjang (*Vigna catjang* Walp.) showed a high degree of resistance and the Arlington variety was almost immune. On these resistant forms, very minute lesions, in fact scarcely visible specks or dots, were noted the fourth day but were more or less healed over and almost invisible by the eleventh day after inoculation.

No hosts other than the *Vigna* species have been found susceptible. The similarity of the disease to cucumber scab in its preference for young tissues suggested a relationship, but two attempts to infect cucumber seedlings were a total failure while with the same inoculum abundant infection of cowpea seedlings was obtained. Failure likewise attended similar inoculation tests with field pea, hyacinth bean, and four varieties of garden bean.

Field observation would indicate that the disease needs to be feared principally on the Early Buff variety of cowpeas. In 1924 the following varieties were arranged in small parallel plots as follows: Whippoorwill, Blackeye, Iron, Early Buff, catjang (*Vigna catjang*), Groit, New Era, and Early Red. In August when the disease was very conspicuous on the Early Buff, the vines from the adjacent rows of the Iron cowpeas and catjang peas were intermingled with the Early Buff plants and yet no lesions could be found on these nor on any of the other varieties.

The relation of the fungus to the host tissue has not been carefully studied. Microscopic examination of inoculated leaflets cleared in chloral hydrate and stained in safranin or Delafield's haematoxylin indicates that germ tubes from the spores do not enter the stomata. Sections through hypocotyl and pod lesions show that there is a collapse of the host cells in the invaded region attended by an accumulation of the dark mycelium and a prompt sporulation of the parasite on the surface. Pod lesions are often accompanied by a limited hyperplasia of the underlying parenchyma cells. Hypocotyl and epicotyl lesions frequently involve the entire thickness of the cortex and occasionally cause a longitudinal fissure extending well into the pith.

SEED TRANSMISSION

Because of the very abundant pod infection (Pl. XXI, figs. A to D), the fungus may readily gain access to the seed both by direct mycelial invasion and by surface contamination with spores, sporophores, and mycelium during threshing. A case was previously described in which the fungus had invaded a seed under a pod lesion and was sporulating on the seed. Since, in its mode of germination, the cowpea seedling usually carries the seed coat up into the air on the cotyledons and frequently carries it higher still on the first leaves (Pl. XXIII, fig. E), any organism on or in the testa may readily gain access to the growing portions of the seedling.

In order to test the possibility of seed transmission of this fungus, seeds collected from under the lesions in diseased pods harvested one month previously were sown in pots of sterilized soil in the greenhouse. When these seedlings were 13 days old and the majority were still bearing their seed coats aloft, they were kept moist four days in a humid compartment in order to afford the fungus favorable conditions for infection. Within one week 11 of the 51 seedlings, or 21 per cent, were showing infection in the shape of elongated, sunken, hypocotyl lesions, from 5 to 15 mm. in length, some with the fungus sporulating at the center. In three cases the lesion extended downward from a cotyledon scar (Pl. XXIII, fig. F) and in two of these cases the cotyledon attached at this scar was infected and at its base bore the fungus in a sporulating condition (Pl. XXIII, fig. F). Lesions which occurred at the ground line or midway of the hypocotyl (Pl. XXIII, figs. E and G) had in some cases penetrated so deeply as to cause the seedling to bend over. In fact a number of the seedlings were soon killed by these primary lesions.

The causal fungus was isolated from five of these seedlings by pouring plates from the spores produced on the hypocotyl lesions and also in one instance from the spores produced on the base of an infected cotyledon. One of these cultures from a hypocotyl lesion was used in successful inoculation tests. These tests prove that the fungus is carried with the seed. The selection of seed from apparently healthy pods did not entirely eliminate the disease, however, as 4 out of 131 seedlings grown from such seed developed hypocotyl lesions. This is attributed to surface contamination of the seed.

Among seed lots of a number of varieties planted in pots in March, a few cases of what was in all probability seed-borne infection occurred in the Large Blackeye variety. The viability of the fungus at this date would indicate that it may very readily persist from season to season in the seed.

SUMMARY

The Cladosporium spot disease of cowpeas is characterized by blackened scabby spots on the pods, sunken purplish spots on the peduncles and stems, and small blackened spots on the leaves and bracts. The causative fungus has been isolated and its pathogenicity proved. It has been designated as *Cladosporium vignae* n. sp. The Early Buff is the most susceptible variety and is the only one on which the disease has been observed in the field. However, greenhouse inoculation tests were successful on 14 other varieties, of which the Progressive White proved to be very susceptible and the varieties, Early Black and Taylor and especially the Arlington, showed a high degree of resistance. *Vigna sesquipedalis* proved susceptible, while *Vigna catjang* showed high resistance. Only young growing tissues are susceptible to infection. The disease is seed-transmitted.

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DESCRIPTION OF PLATES

PLATE XXI

FIGS. A to D. Types of pod infection, Cladosporium spot of Early Buff cowpea. A and B are heavily infected pods somewhat distorted in their development by the presence of the lesions. Near the lower end of figure A, very small lesions may be seen. D shows large lesions with dark borders and tan centers.

FIG. E. Colonies of *Cladosporium vignae* 10 days old. Poured plate culture from spore suspension, potato dextrose agar. Enlarged × 2.

PLATE XXII

FIG. A. Three young peduncles showing the sharply sunken, purplish lesions. Enlarged × 2.

FIG. B. Lesions on older peduncles.

FIG. C. Lesions on a very young pod causing crooks and inhibiting the normal enlargement.

FIG. D. Lesions near the growing point of a stem and on petiole and leaflets.

FIG. E. Lesions on a young pod under which inhibition of seed development has resulted.

FIG. F. Conspicuous dry scabby lesions on early mature pod. Enlarged × 2.

PLATE XXIII

FIG. A. Small, inconspicuous lesions on young leaf, as a result of natural field infection. Enlarged × 1½.

FIG. B. Very small, blackened lesions on the first leaf of a seedling of the less susceptible New Era variety as a result of atomizer inoculation made 6 days previously. These lesions did not increase further in size.

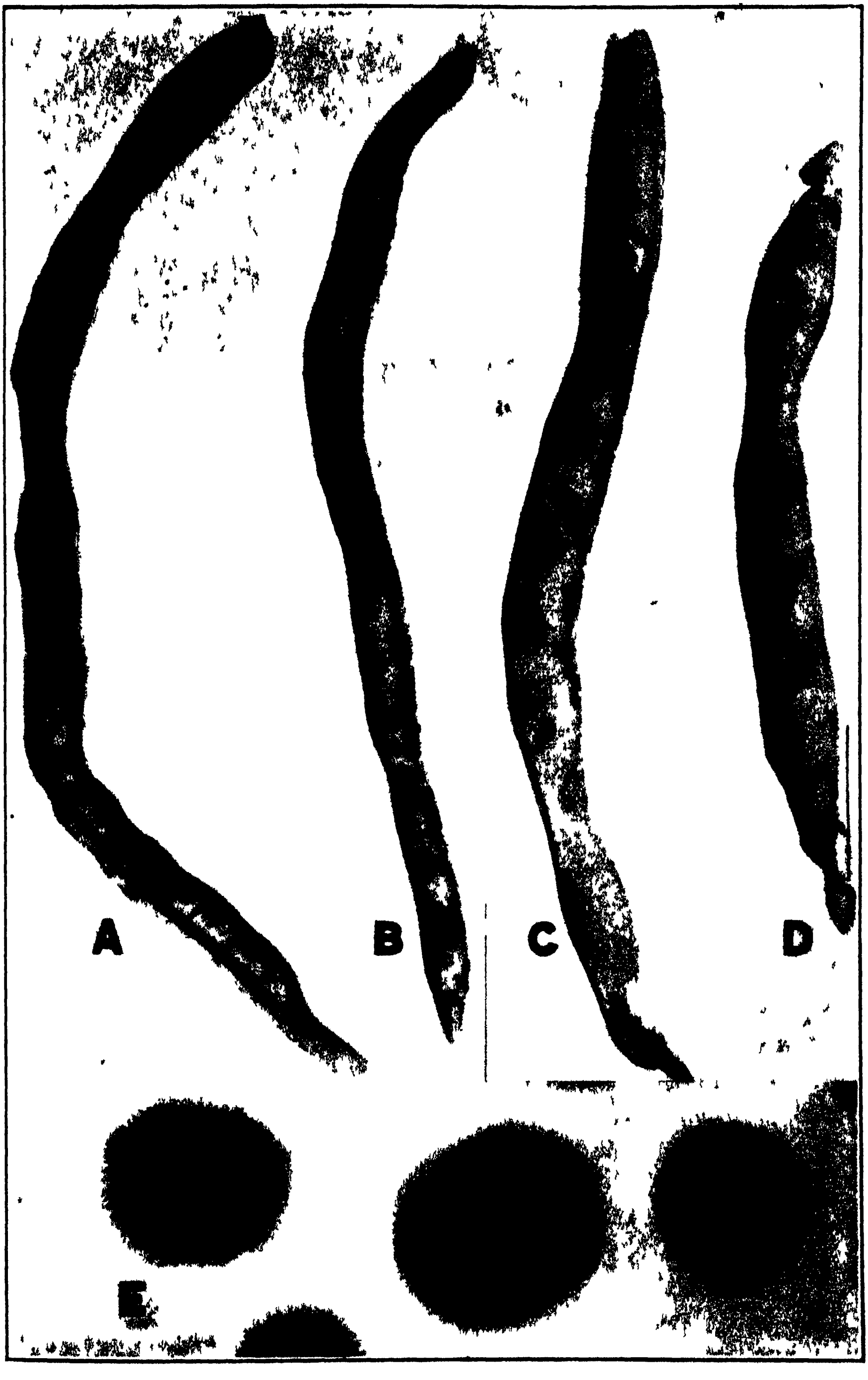
FIG. C. First compound leaf of Early Buff seedling showing large lesions resulting from atomizer inoculation made 16 days previously when the leaf was unfolding. Enlarged × 2.

FIG. D. Lesions on first leaf of Early Buff seedling inoculated after the leaf was full grown. Enlarged × 2.

FIG. E. Seedling showing primary lesion on hypocotyl as a result of seed-borne infection. The lesion penetrated so deeply as to cause the hypocotyl to break at that point. The seed coat has been carried up on the first leaves. Enlarged × 1½.

FIG. F. Seedling showing lesion at the cotyledon scar. The cotyledon to the left, which was attached to this scar, is infected at its basal end and the fungus is sporulating thereon. Enlarged × 1½.

FIG. G. Primary lesion on hypocotyl. Enlarged × 2.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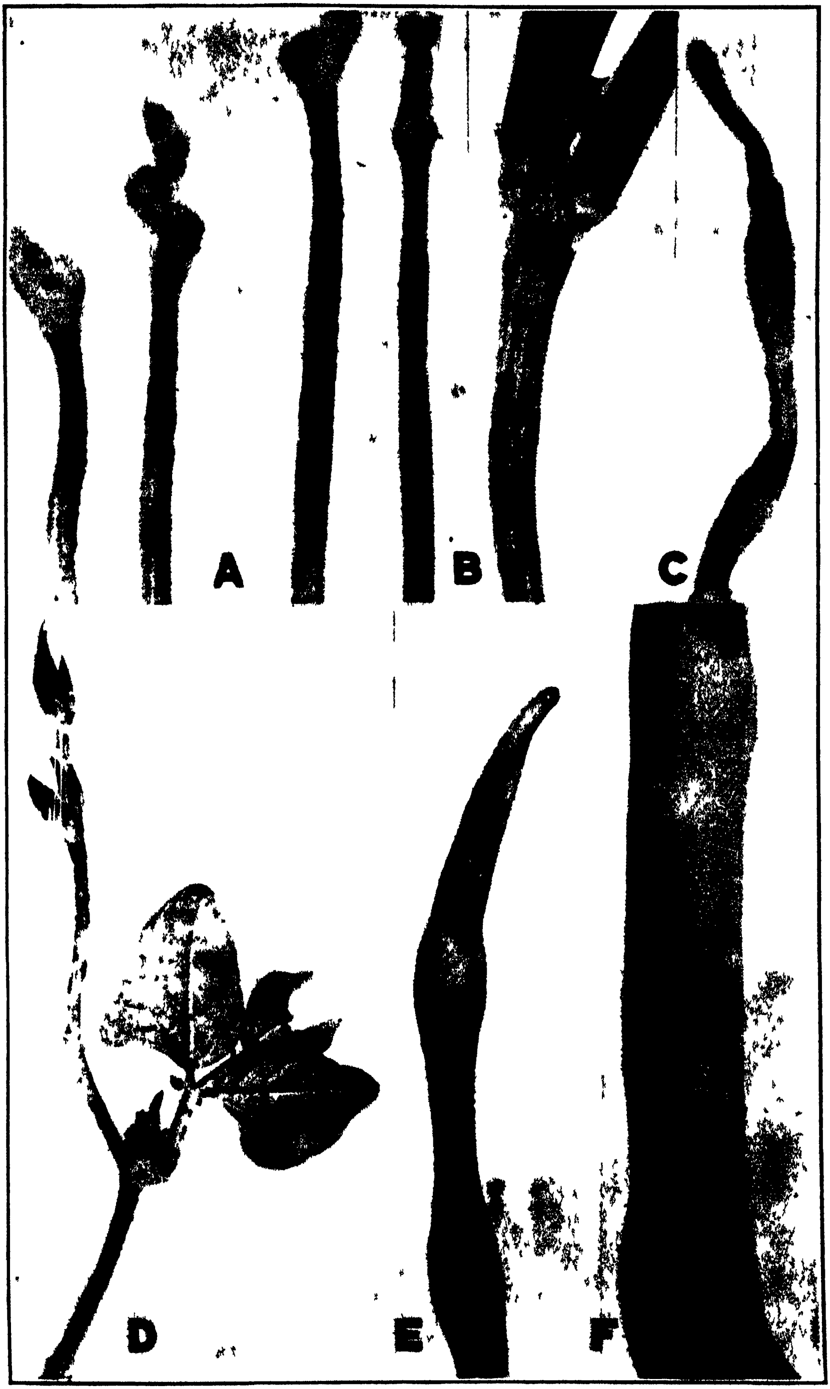
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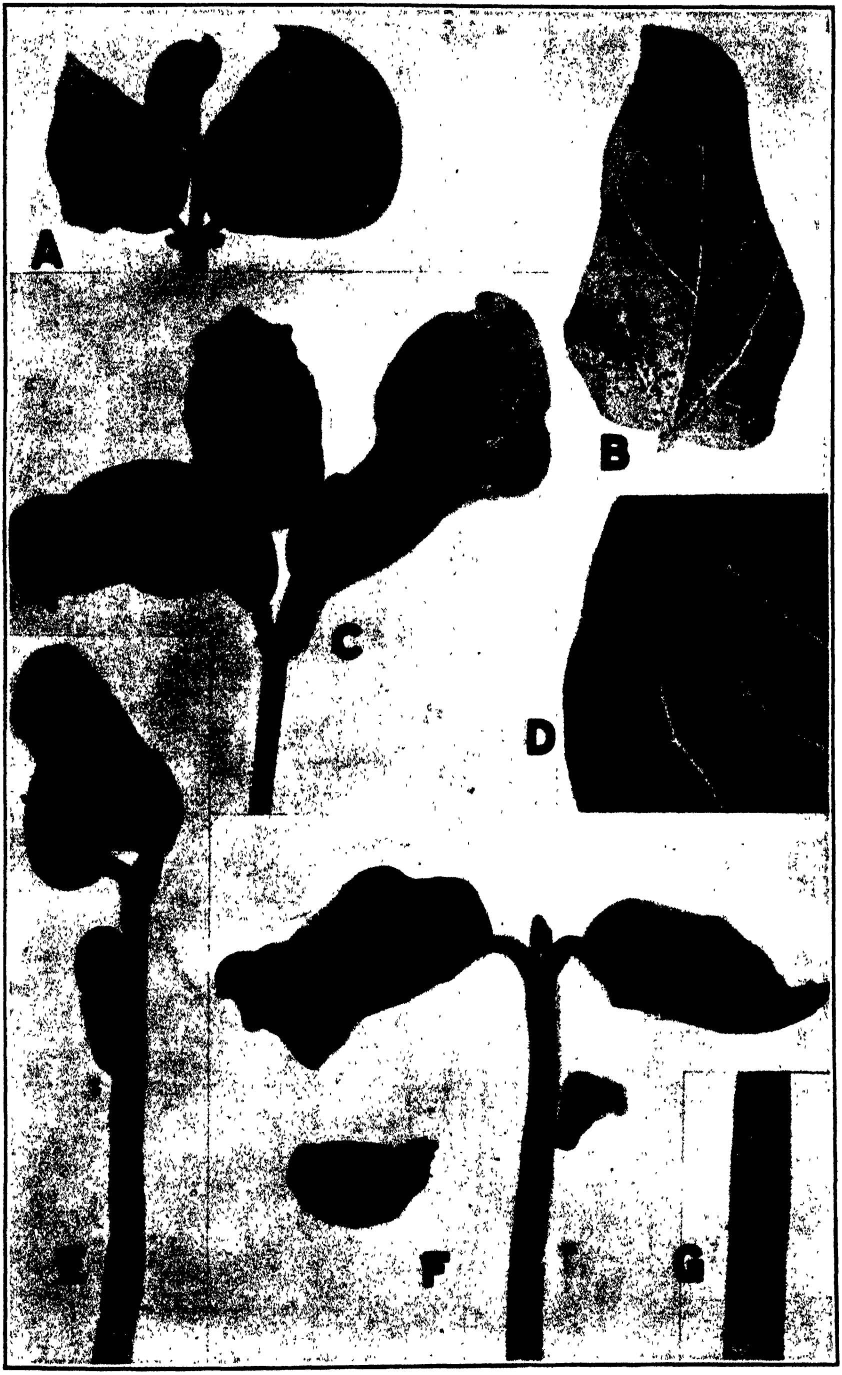
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RELATION OF GROWTH OF HELMINTHOSPORIUM SACCHARI TO MAINTAINED TEMPERATURES[1]

F. F. HALMA AND H. S. FAWCETT

It is generally recognized that the growth of parasitic fungi is greatly influenced by temperature. Among many recent investigations which have a bearing on this question a few of the recent ones may be mentioned.

According to Dickson (1), soil temperature is the most important single factor determining the extent of seedling blight of wheat and corn. Rosenbaum and Ramsey (2) found that the development of blackleg of the potato is closely correlated with temperature and precipitation. Stevens (3) has shown that the rate of increase in diameter of cankers of chestnut blight varies with the temperature. Edson and Shapovalov (4) found a certain degree of correlation between temperature relations of some potato fungi in pure culture and their geographical distribution and seasonal occurrence. Lauritzen and Harter (5) established the fact that of the two species of *Rhizopus* responsible for soft rot in sweet potatoes one is responsible for decay at a higher and the other at a lower temperature. Walker and Jones (6) believe that the regional distribution of onion smut in the United States is conditioned by the soil temperature during the seedling stage of the plant's growth. Fawcett (7, 8) has suggested that temperature conditions may be among the most important factors limiting the prevalence of diseases due to *Phomopsis citri* Faw. and *Cladosporium citri* Mass.[2] In another investigation (9) he found that the growth of four different fungi were characteristically affected by temperature.

From the literature cited above it is evident that a knowledge of temperature relations is of importance to the investigator working with any fungus. It is for this reason that the results of this investigation are placed on record.

Mr. H. A. Lee, of the Hawaiian Sugar Planters' Association, wishing to ascertain the temperature relations of *Helminthosporium sacchari*, sent cultures to this laboratory where facilities for such an investigation are available. From these cultures several stock cultures were made by transferring small pieces of mycelium to petri dishes containing standard nutrient agar. This constituted the source of the material used.

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[1] Paper No. 124, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] This fungus is being described by Miss Anna E. Jenkins as *Sphaceloma fawcettii*.

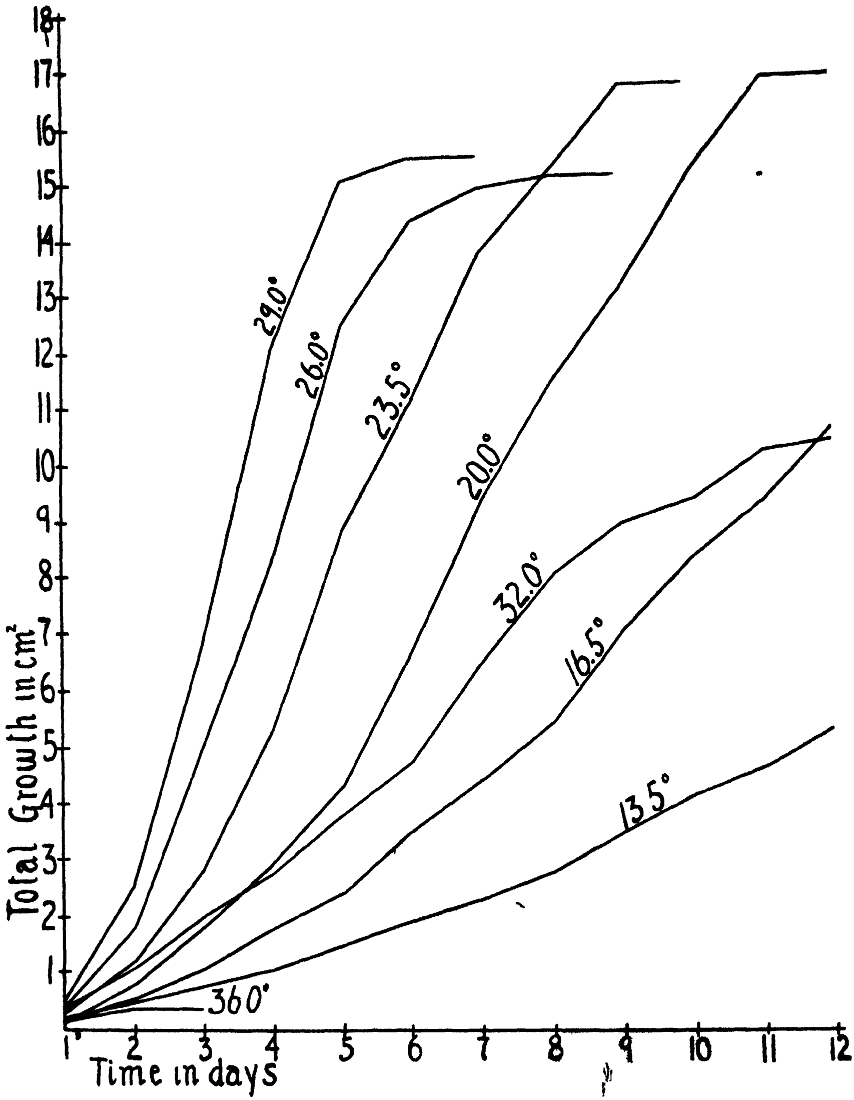

FIG. 1. Graph showing the growth of *Helminthosporium sacchari* at different temperatures with time.

The fungus was grown in standard nutrient agar and in standard bouillon. Each petri dish contained 15 cc. of the former and each Erlenmeyer flask 100 cc. of the latter. The hydrogen-ion concentration of the bouillon was pH 6.8. A disk 2 mm. in diameter, cut out near the advancing margin of the mycelium of a stock culture, was introduced into each of the petri dishes and flasks. These were immediately placed in the various temperature chambers.

For each temperature ten petri dishes and ten flasks were used. Eight different temperatures were employed as follows: 13.5, 16.5, 20.0, 23.5, 26.0, 29.0, 32.0, and 36.0° C. These temperatures with the exception of 36° C. (which was maintained in an incubator) were maintained by means of an apparatus similar to that described by Livingston and Fawcett (10).

The increment in the diameter of the mycelial mass was measured daily. From these data the area was calculated and this was taken as the index of growth. The mycelium in the bouillon was allowed to grow for ten

TABLE 1.—*Growth of Helminthosporium sacchari on standard nutrient agar at different temperatures*

Days	Temperature in degrees Centigrade							
	13.5	16.5	20.0	23.5	26.0	29.0	32.0	36.0
1	5.1[a] 0.2	4.6 0.2	4.8 0.2	6.5 0.3	7.4 0.4	8.6 0.6	6.9 0.4	5.2 0.2
2	7.6 0.5	8.6 0.6	10.1 0.9	12.7 1.2	15.4 1.9	18.2 2.6	12.1 1.1	7.2 0.4
3	10.1 0.8	11.8 1.1	15.2 1.8	19.1 2.8	25.1 4.9	29.7 6.9	16.0 2.0	Growth ceased
4	12.1 1.1	15.2 1.8	19.2 2.9	26.1 5.3	32.6 8.3	39.2 12.1	18.8 2.8	
5	14.0 1.5	17.5 2.4	23.7 4.4	33.6 8.9	40.1 12.6	43.8 15.1	21.9 3.8	
6	15.7 1.9	21.0 3.5	29.2 6.7	37.8 11.2	42.8 14.4	44.5 15.5	24.6 4.8	
7	17.3 2.3	23.6 4.4	34.6 9.4	41.9 13.8	43.7 14.9	Growth ceased	28.9 6.5	
8	19.1 2.8	26.9 5.7	38.2 11.5	44.3 15.3	44.0 15.2		32.3 8.1	
9	21.1 3.5	30.1 7.1	41.1 13.2	46.3 16.8	Growth ceased		33.8 9.0	
10	23.1 4.2	32.6 8.3	44.2 15.3	Growth ceased			34.7 9.4	
11	24.5 4.7	34.6 9.4	46.4 16.9				36.3 10.3	
12	25.9 5.3	36.9 10.7	Growth ceased				36.5 10.4	

[a] Upper number = diameter of mycelium in mm.; lower number = area of mycelium in cm^2.

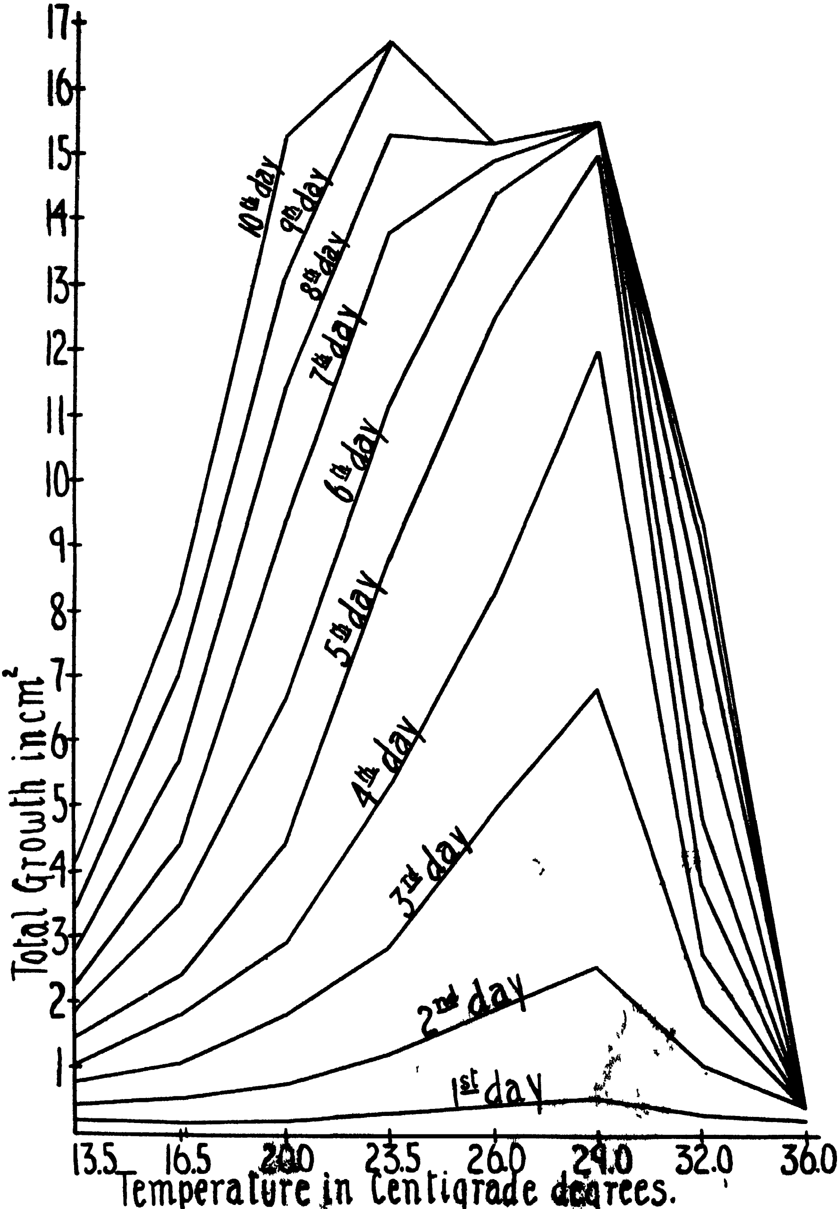

FIG. 2. Graph showing the rate of growth of *Helminthosporium sacchari* at different temperatures.

days, then filtered through "Genuine Whatman Filter Paper" which had previously been weighed. The mycelium was thoroughly washed with distilled water, then air dried in a closed room for one week. The total net weight of the air-dried mycelium of each set was taken as a measure of growth. The hydrogen-ion concentration of the bouillon both before and at the completion of the experiment was determined by comparing it with standard buffer solutions.

GROWTH IN STANDARD NUTRIENT AGAR

Table 1 and figures 1 and 2 show that, with the exception of 36° C., the fungus was able to grow in all the temperatures employed. It may be

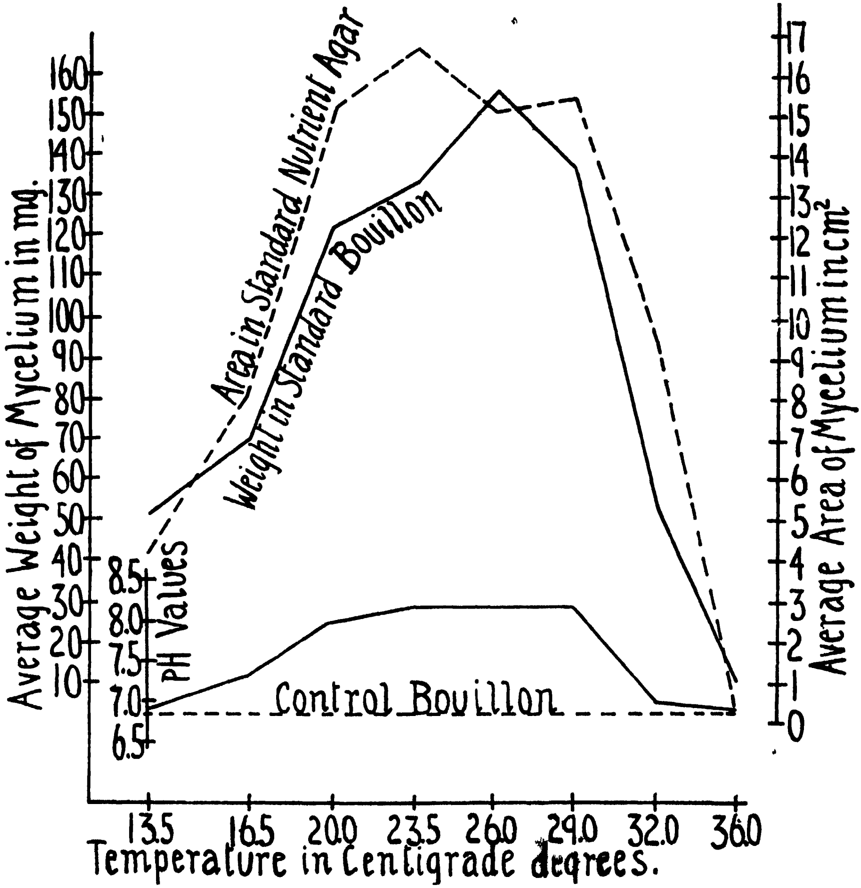

FIG. 3. Graph showing the amount of growth made by *Helminthosporium sacchari* in 10 days in solid and liquid media and the change in the hydrogen-ion concentration of the bouillon.

mentioned in this connection that the organism was not destroyed at 36° C., as growth took place promptly when the petri dishes were transferred to room temperature after remaining at 36° C. for ten days.

Under the condition of the experiment the optimum temperature for *Helminthosporium sacchari* is seen to lie between 20° and 29° C. However, the most rapid growth took place at 29° C. until it came to a stop on the seventh day; then the optimum gradually shifted to the lower temperatures until the twelfth day, when growth ceased in the optimum range. It is of interest to note that in all the four temperatures lying between 20° and 29° C. the final average area of the mycelium was practically the same. In no case did the mycelium occupy more than one-third of the area of the medium.

GROWTH IN STANDARD BOUILLON

The data given in table 2 and figure 3 confirm the results obtained in nutrient agar, namely, that the optimum range for this fungus lies between 20° and 29° C. An additional point of interest is that the reaction of the bouillon changed with the amount of growth. The pH value of the bouillon remained practically the same at temperatures at which only slight growth took place; but in the optimum range the bouillon became distinctly alkaline (see table 2).

TABLE 2.—*Growth of Helminthosporium sacchari in standard bouillon at different temperatures*

Temp. C°	Average wt. of air-dried mycelium in mg.	pH of bouillon at end of 10th day
13.5	51.0	6.9
16.5	75.0	7.3
20.0	126.0	8.0
23.5	132.5	8.2
26.0	157.0	8.2
29.0	136.5	8.2
32.0	53.5	7.0
36.0	10.0	6.9

SUMMARY

Helminthosporium sacchari was grown in standard nutrient agar and standard bouillon at eight different temperatures. Under the conditions of the experiment the optimum temperature for this fungus was found to lie between 20° and 29° C. The optimum, however, in nutrient agar remained constantly at 29° C. for the first seven days, when growth ceased at that temperature. Within the optimum range (20° to 29° C.) the reaction of the bouillon became distinctly alkaline, whereas only a slight change in pH occurred at those temperatures at which the growth was slight.

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HIGH EVAPORATION: A PRECURSOR AND A CONCOMITANT OF WESTERN YELLOW TOMATO BLIGHT

MICHAEL SHAPOVALOV

WITH SIX FIGURES IN THE TEXT

It is generally recognized that climatic conditions may either favor or arrest the development of plant diseases, but not all climatic factors involved have received the recognition which they deserve. As bacterial and fungus parasites are the principal causative agents of the diseases, a humid atmosphere accompanied by a medium to high temperature appears to be most favorable for the development of many troubles. Consequently, the attention of investigators was mainly given to temperature, precipitation, and higher humidities. The rôle of evaporation, especially of high rates of evaporation, has been decidedly less studied and less understood. However, the connection between the rate of evaporation and certain plant diseases is apparent and the need of more complete evaporation data for various agricultural sections is already being felt.

In the case of western yellow tomato blight,[1] a certain seasonal march of evaporation means a definite progress of blight in the same season. This disease causes serious annual losses to the growers over a large territory extending west of the Rocky Mountains to the Pacific coast and from British Columbia to the west coast of Mexico, but the actual annual damage fluctuates according to seasonal conditions. The summer of 1924 was marked by a particularly severe outbreak of western blight in a number of widely separated regions of the West and for this reason is especially interesting. This outbreak correlates with an unusually high evaporation in all those sections in which it occurred, as will be seen from the following analysis.

Reports from Utah show that the damage from blight in that state last summer was the most serious since the epidemic of 1905 and by far exceeded the average annual damage from the disease in the state. It is generally estimated that the last year's loss ranged between 30 and 35 per cent of the state crop, while the average annual loss probably does not exceed 5 per cent.[2] The toll taken by this blight in the state of Washington was even more severe. It reached an average of probably 50 per cent, and many patches were observed where 75 per cent of the plants were affected.[3] Similar conditions also prevailed in the southwestern corner of Idaho where climatic conditions are identical with those of the adjacent portions of Washington. Tomato-growing in California is scattered in climatically different and even contrasting regions, and the amount of the disease in these regions varied from practically nothing in humid sections near the coast to nearly 100 per cent in localities with a high rate of evaporation. The severest attack of the disease in the state last summer occurred at Shafter (near Bakersfield) where, in a field of 1700 plants, all but 16 died from blight by the end of June, and only 3 were left unaffected by the end of July. Also quite severe loss was suffered by tomato growers in the section surrounding Riverside. Nearly 40 per cent of plants blighted in 1924 in the writer's experimental field at Riverside, in which less than 2 per cent blighted in 1923, the same variety (Stone) being used both years. Finally, it is very significant that the first definite report of the occurrence of western tomato blight east of the Rocky Mountains, in the state of Kansas,[4] was received in 1924. The disease did not appear to be very severe there, as only a few plants here and there in a patch blighted, but it was scattered in various parts of the state. Heretofore the reports were practically confined to the territory west of the Rocky Mountains, with a few cases reported from as far as New Mexico.[5]

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[1] The term "blight" is frequently used in this paper for the sake of brevity, but in every instance it has reference to western yellow blight, a disease characterized by a general yellowing and a pronounced rigidity of the plant, torsion of the leaves, purpling of the veins and cessation of growth, followed by death and accompanied by a progressive root decay.

[2] Plant Disease Reporter 8: nos. 6 and 10. U. S. Department of Agriculture, Washington, D. C. 1924.

It is very illuminating to compare these reports of the unusually severe outbreaks of the tomato blight with the weather conditions in certain representative sections of the states just mentioned. It will be seen (Figs. 1–4)[6] that the rate of evaporation in 1924 was in every case above the average of a number of preceding years. This condition was due mainly to a lower relative humidity and a greater wind movement. The relative humidity at Salt Lake City in 1905 was much below the average, indicating a high evaporation, which explains the fact that that year is remembered in the state as a severe blight year. The departures of mean temperatures are not significant and not uniform for various blight sections. The same may be said of the precipitation means for Utah and Washington; but in Wichita, Kans.,

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[3] Plant Disease Reporter 8: no. 10. U. S. Department of Agriculture, Washington, D. C. 1924.

[4] Reported by Dr. L. E. Melchers, of the Kansas State Agricultural College.

[5] Plant Disease Reporter 8: no. 6. U. S. Department of Agriculture, Washington, D. C. 1924.

[6] The data for all figures except figure 4 were obtained from the U. S. Weather Bureau publications, the Monthly Climatological Data and the Reports of the Chief. The evaporation data for figure 4 were secured by the writer by means of porous porcelain cup atmometers.

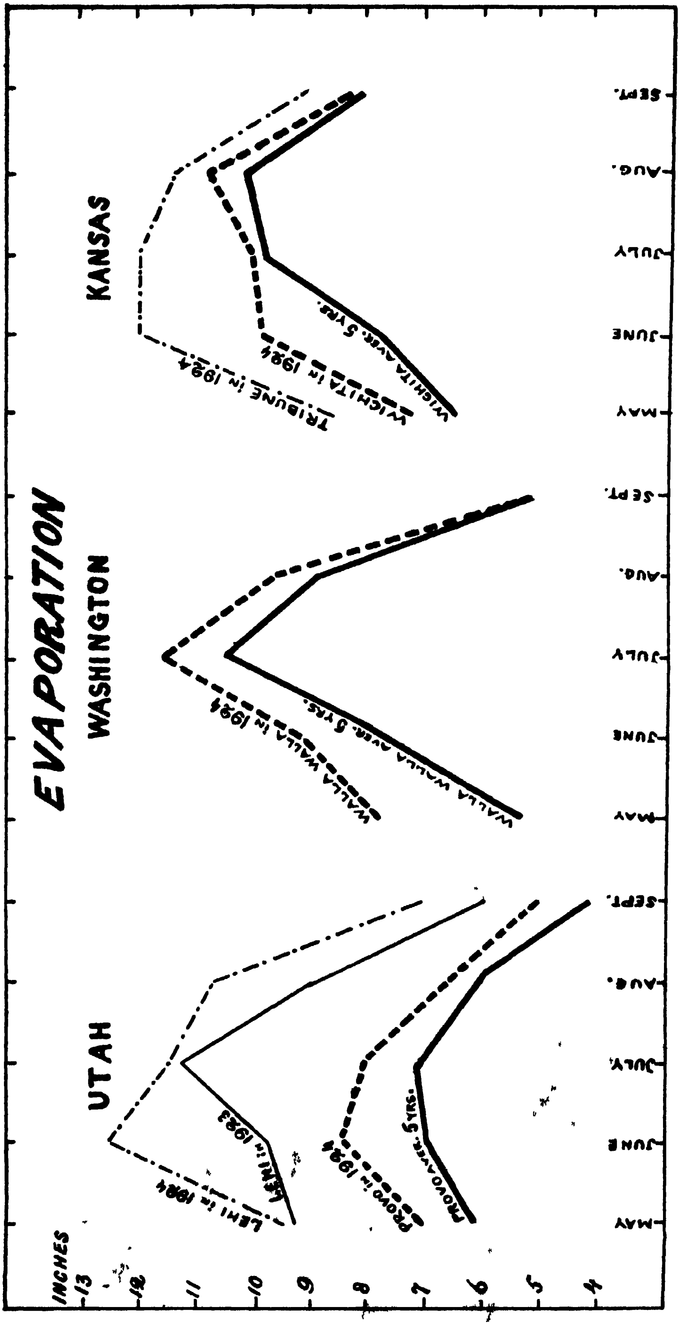

FIG. 1. Showing an excessive rate of evaporation in 1924 in some of the sections from which outbreaks of western yellow blight were reported.

it not only fell considerably below the normal during the month of June, but even reached the level of arid sections of Utah and Washington. This extraordinary deficiency in rainfall at Wichita (and other tomato-growing sections of the state as well) may have played an important rôle, emphasizing the effect of the conditions causing high evaporation and thus making the occurrence of western yellow blight in the state possible.

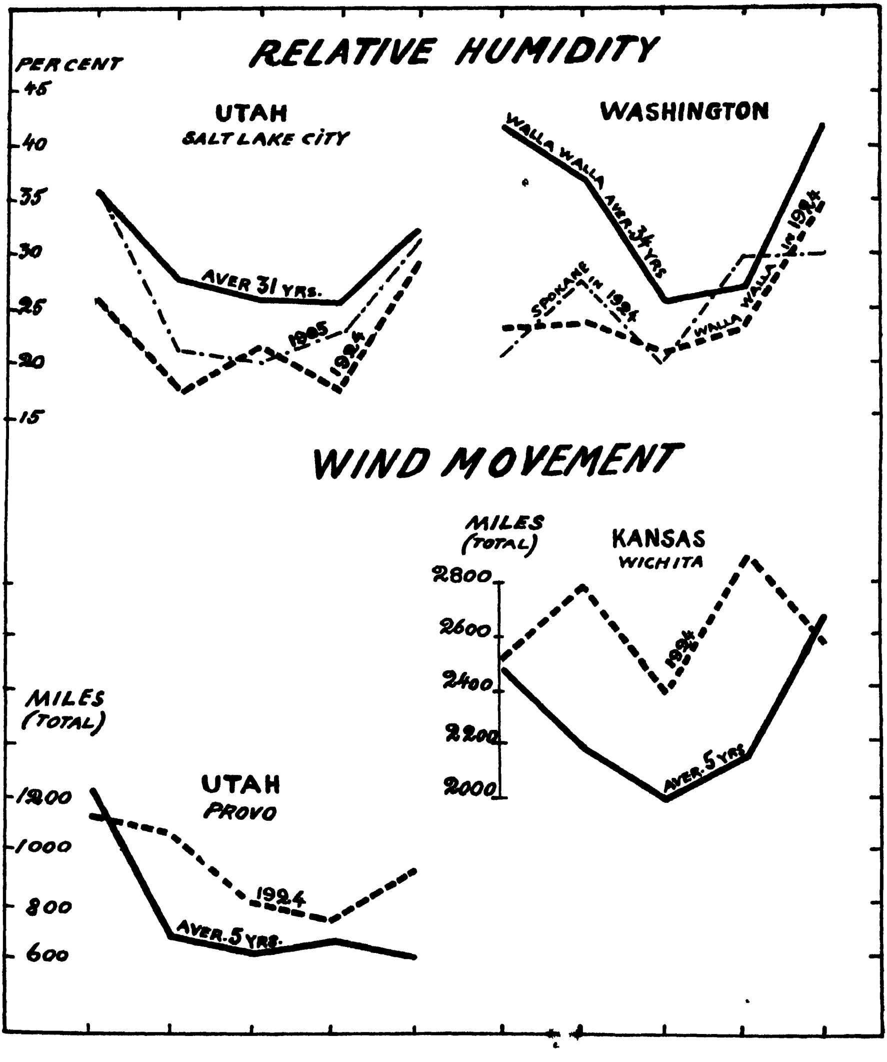

FIG. 2. Showing unusually low relative humidity and high wind movement in 1924 in several localities where western yellow blight was particularly severe.

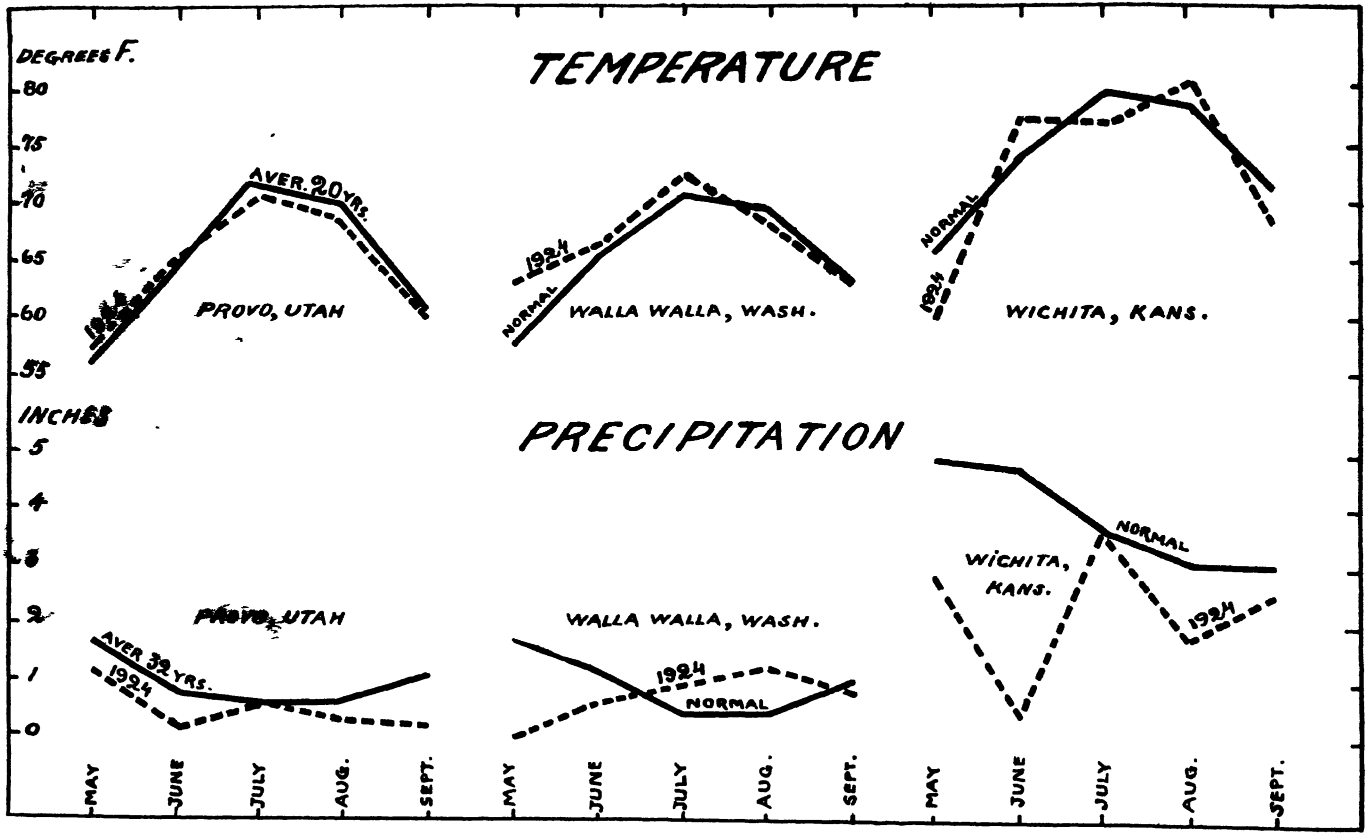

FIG. 3. Showing temperature and precipitation in certain blight sections in 1924 as compared with averages and normals. It can be seen that monthly fluctuations are not uniform and do not correlate with the progress of the disease.

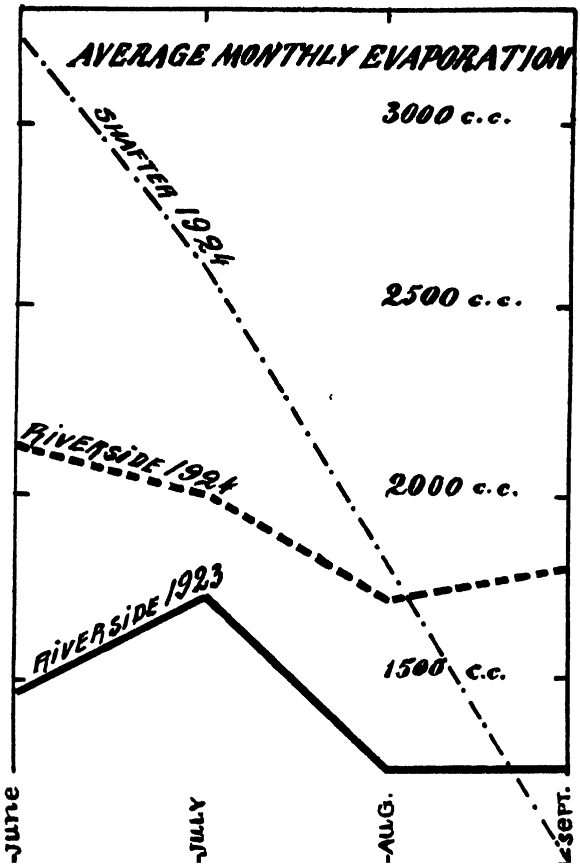

FIG. 4. Evaporation on experiment plots at Shafter, Calif., and Riverside, Calif., as related to the progress of western yellow blight. There was 1.7 percent of blight at Riverside in 1923, 39.1 percent in 1924, and practically 100.0 percent at Shafter in 1924.

The most striking correlation between the amount of blight and the rate of evaporation was observed on the experimental plots at Shafter, Cal., and at Riverside, Cal., where counts of blighted plants were made throughout the season. The disease developed slowly and in very small amounts with the lowest evaporation curve at Riverside in 1923. It was more severe in 1924 when the evaporation was higher, but the most serious attack and in much shorter time developed with the highest rate of evaporation at Shafter in 1924.

The comparison of the percentages of blight at Riverside and at Shafter in 1924 indicates that the rate of evaporation is not only concomitant with the severity of blight in different seasons in the same locality, but also cor-

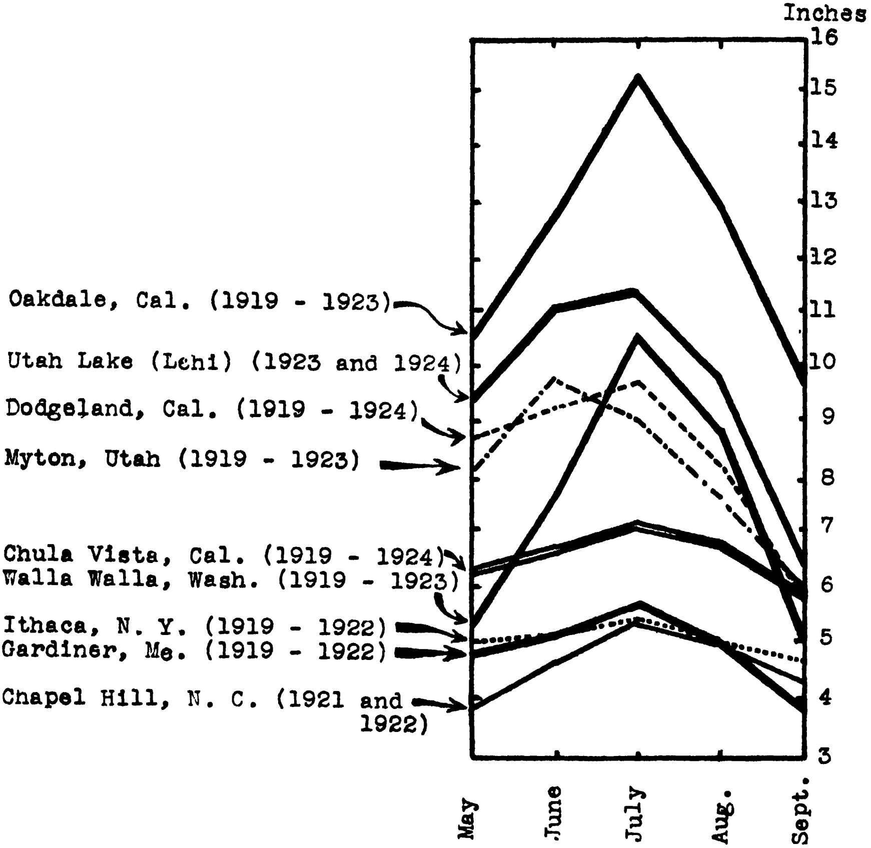

FIG. 5. Evaporation (average monthly amounts).

relates with its geographical distribution. This may be illustrated by comparing various blight and non-blight sections of the West. The blight problem is more or less serious south of Lake Okanagan and in the state of Washington, through the valleys of Wenatchee and Yakima, and in the Clarkston-Lewiston district on the Snake River, also at The Dalles, Ore., in the territory east of the Great Salt Lake of Utah, in the Sacramento, San Joaquin and San Fernando Valleys of California, near Riverside, near Sinaloa (Los Mochis), and Culiacan, Sin., Mexico, and, in general, in the western irrigated territory. It is of slight or no importance near Vancouver, at Puget Sound, in the vicinity of Portland, in the Bay Region of San Francisco, in the coastal trucking sections of Los Angeles, Orange, and San Diego counties, near Mazatlan, Sin., Mexico, and, in general, in the eastern United States. Figures 5 and 6 give a comparison of the rates of evaporation and the relative humidity of these blight-free and blight-infested geographical regions. The greatest difference occurs during the summer months of June, July, and August, which are also the principal blight months. There is a regrettable scarcity of localities (especially within the agricultural territory) for which evaporation records are taken. Out of nine such localities (given in Fig. 5) lying in or near tomato sections, five are blight sections and have the average monthly evaporation during the blight period above 7 inches, and the remaining four are blight-free sections with the average monthly evaporation for the same period at or below 7 inches (Chula Vista being at the top of this group). Relative humidity records are available for a greater num-

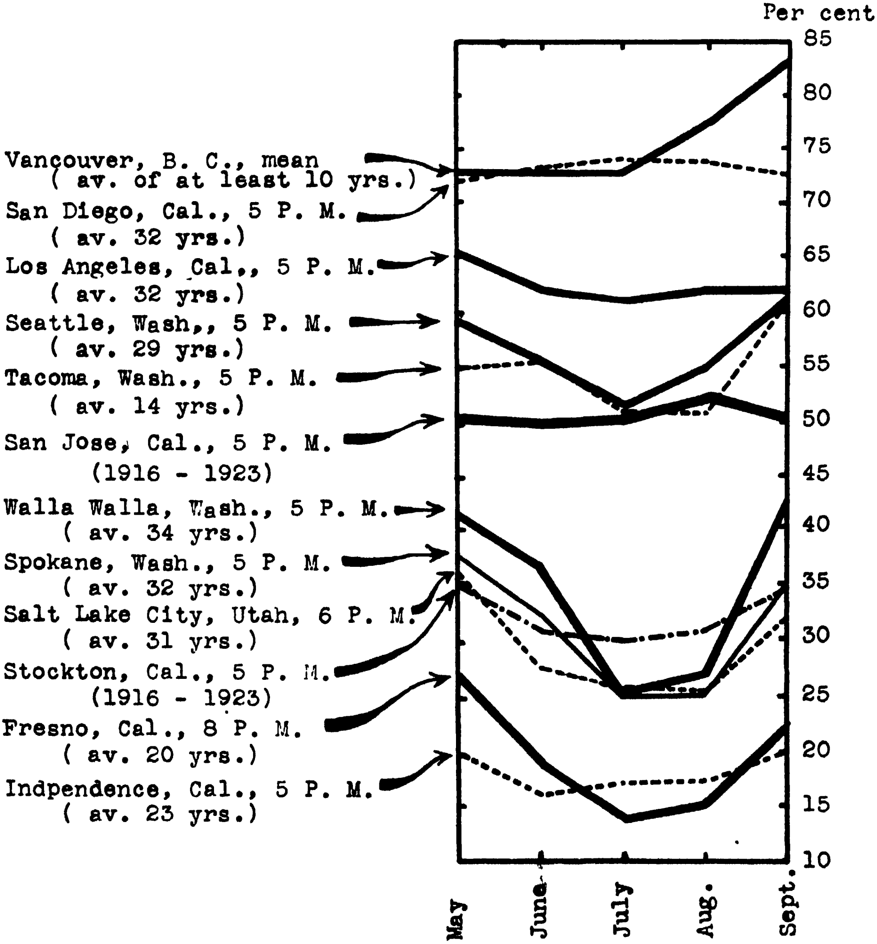

FIG. 6. Mean relative humidity (averages).

ber of stations, and figure 6 gives the humidity curves for six from each of the two blight-contrasting groups of localities. Blight areas during the principal blight period seem to have the average monthly humidity below 35 per cent, while non-blight areas about or above 50 per cent. (San Jose, which is a moderate blight section, approaches this latter group during the second part of the summer.) The extremes for each group are to be found in the San Joaquin valley for the former, and near Vancouver and San Diego for the latter. High evaporation is not only attendant on, but also foreruns severe spells of blight, especially at the outset of the season. This was found to be true in 1924 both at Riverside and at Shafter. Detailed weekly observations at these places from the time the plants were set out in the field show very distinctly that the first serious waves of the disease were preceded by marked rises in the rate of evaporation.

Not only the progress of blight is arrested, but a phenomenon of an entirely different order takes place when the evaporation drops to a low level for a more or less protracted period. In this case such blighted plants as are not in the last stage of the disease show a recovery which, although ordinarily not complete and seldom leading to the restoration of the normal state, is nevertheless very significant scientifically. On the Riverside experimental plots in 1924 nearly 10 per cent of the total number of blighted plants showed partial recovery (one plant recovered completely) during the month of August, when the evaporation was at its lowest ebb for the four summer months.

It is thus quite apparent that the evaporation data are indispensable for the conduct of western yellow tomato blight studies as well as for a better understanding of the normal growth of the tomato plant, as the conditions which favor high rate of evaporation also favor severe outbreaks of this blight. This was exceedingly well illustrated by the condition which prevailed during the 1924 season.

It is most likely that the evaporation will be found to have an important bearing on the disease problem of many agricultural crops when ecological relationships of these latter are better understood. Adequate evaporation records would both facilitate such understanding and stimulate new efforts towards it. Consequently, it is very urgent that more weather stations, especially those located in agricultural sections, gather evaporation data along with the data on relative humidity, wind movement, sunshine, and other factors influencing the rate of evaporation.

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FUMIGATION INJURY OF WATERMELONS

G. B. RAMSEY[1]

WITH ONE FIGURE IN THE TEXT

During the past season, the Food Products Inspectors of the Bureau of Agricultural Economics were called upon to inspect several cars of California watermelons for a peculiar type of injury that was causing extensive damage in certain cars. The writer's attention was first called to this trouble by Mr. R. L. Ringer, of Portland, Oregon. At one time he inspected five cars in which every melon was blemished by blisters and sunken areas which resembled anthracnose, but upon inquiry of the authorities at place of origin it was found that neither vines nor fruits in the field showed evidence of anthracnose. The question was then raised as to whether the fumigation with formaldehyde, as required by the quarantine on account of the "Hoof and Mouth" disease, might not be responsible for this damage. Shipments into the state of Washington were required to be fumigated, but Oregon did not require fumigation, and this fact led to a striking demonstration which points directly to the formaldehyde fumes as the damaging agent. A stock car of melons arrived in Oregon in perfect condition, but before being sent on into Washington, they had to be fumigated. The melons were therefore transferred to a refrigerator car that could be closed up tightly and were fumigated and sent on their way to Vancouver, Wash. On arrival, forty-eight hours later, every melon was blistered and pitted. Over fifty cars of California watermelons are known to have been seriously affected by this type of injury.

Mr. B. A. Harrigan, Horticultural Commissioner at El Centro, California, writes regarding this trouble:

> The only places we experienced pitting and sunken areas were in melons that sat in the sun too long after having been picked. We found that melons picked during midday and left in the field would show considerable damage in five hours. Judging from past experience I believe that watermelons will show no damage, from fumigation, if they are picked before ten o'clock in the morning and after four o'clock in the afternoon and moved out of the field.

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[1] Contribution from the Research Laboratory on Market Diseases of Vegetables and Fruits; United States Department of Agriculture, Bureau of Plant Industry, and the Botany Department, University of Chicago, cooperating.

The writer has seen similar lesions caused by fumigation, in other products, so it seemed not unreasonable to think that this particular watermelon trouble might be associated with fumigation. With a view to testing the possibility of reproducing this injury, a perfectly smooth and well-colored melon was placed in a closed transfer chamber and fumigated with formaldehyde. The formula used was the same as that recommended for the car-lot treatment of fruits and vegetables for control of the "Hoof and Mouth" disease, viz.: potassium permanganate 16 ozs., formaldehyde 20 ozs., per 1,000 cu. ft. of air space, for a period of four hours. The glass walls of the transfer chamber permitted the observer to see the melon without liberating any of the fumes. At the end of the four-hour period, no changes were noted in the melon. The door of the transfer chamber was then opened momentarily in order to detect and determine to some degree the strength of the fumes by the odor. The chamber was immediately closed and not opened again for fourteen hours. At the end of this time no injury was apparent, but the door was opened and the remaining fumes allowed to escape. It was not until approximately twenty-seven hours later that definite pits became evident. At this time several small depressions from one-eighth to one-fourth inch in diameter and a few larger ones from one-fourth to one-half inch in diameter were scattered irregularly over the fruit. The larger and deeper lesions were in regions where the melon was slightly bruised or where the cuticle had been rubbed off. These bruises, however, were not apparent as discolorations or depressed areas at the beginning of the experiment.

FIG. 1. Fumigation injury.

This experiment clearly demonstrates that lesions of the type shown in figure 1 can be produced by formaldehyde fumigation. The fact that not all fumigated cars showed injury indicates that a careful study of the technique of application with due attention to environmental factors may be expected to indicate a procedure which is both safe and effective should occasion again arise for fumigating watermelons.

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PYTHIUM INFECTION OF CABBAGE HEADS

CHARLES DRECHSLER

WITH ONE FIGURE IN THE TEXT

In a previous paper[1] dealing with the cottony leak of cucumbers were enumerated some recorded instances in which members of the genus *Pythium* have been found responsible for destruction to plant products. Such cases are deserving of special note, inasmuch as they differ from the instances of damage from damping off and rootlet injury more usually associated with the genus, in that they involve tissues of relatively mature parts. Moreover in certain cases where destruction may be initiated or continued after harvesting, the control problem presented is a different one, consisting, as it does, in the conservation of plant structures under conditions more or less subject to control, rather than in maintenance of the life of the plant or its efficiency as a productive unit by the somewhat indirect and not always effectual means of soil management.

To the instances enumerated may be added a type of decay of cabbage not hitherto described. In July, 1924, the writer received for identification a portion of a cabbage head found on the Washington market by Miss L. McCulloch. Some of the compactly arranged inner leaves were conspicuously water-soaked in appearance, the discolored regions extending somewhat farther along the fleshy midribs than over the thinner lamellae, and evidently proceeding from the base where the modified foliar organs were attached to the affected core. When the affected leaves were separated out, the diseased portions were found to be nearly as firm to the touch as the healthy portions. That this firmness was due mainly to the surface tissues became apparent on dissection, by which means the deeper tissues were revealed as a softened pulpy mass from which water could be freely expressed.

When examined under the microscope the pulpy material was found to consist very largely of abundantly branching, continuous mycelium of the type characteristic of *Pythium* when developing in culture media rich in organic matter, or, for example, in living cucumber or watermelon fruits. And as in these substrata the tissue structures of the host were reduced to disorganized cell walls small in quantity when compared to the abundance of mycelial development. Portions of the material planted on cornmeal agar yielded cultures of a species of *Pythium*, which subsequently were freed of contaminating bacteria.

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[1] Drechsler, Charles. The cottony leak of cucumbers caused by *Pythium aphanidermatum*. In press; to appear in the Journal of Agricultural Research.

A fuller treatment of the morphology and taxonomy of the fungus is reserved for inclusion in a comparative account of the genus *Pythium* now under preparation. It would appear to be a species of the type usually dealt with in the literature as *Pythium debaryanum* Hesse producing subspherical "conidia," smooth oogonia, and oospores, and on suitable artificial media an abundance of cottony aerial mycelium. Hesse's binomial, however, cannot appropriately be applied to it as the sexual apparatus shows marked departures from that distinctive of *Pythium debaryanum,* particularly in the relationship of antheridium to oogonium. When the oogonium is intercalary and borne on the larger hyphae, fertilization is generally accomplished by cylindrical stalked antheridia, of which one or two are usually present, communication being established by a tube entering directly from the septum originally delimiting the two organs. When more delicate hyphae are involved, more frequently a sessile pouch-like outgrowth develops immediately adjacent to the oogonium, and this together with a variable but usually small portion of the hypha is delimited by a septum and functions as an antheridium, communication being established by a tube originating from the pouch-like part and traversing the oogonial wall a short distance from its juncture with the parent filament. Not infrequently antheridia developing from a hypha other than the one bearing the oogonium are encountered, these being either of the "branch" type with the septum at the base of an inflated part, or of a modified "intercalary" type consisting of an intercalary portion of hypha bearing a sessile inflated protuberance from which the fertilization tube is produced, the delimiting septa then being two in number and inserted directly in the hypha. The condition figured by Hesse, and made familiar by numerous text-book illustrations, of fertilization being accomplished by an approximately cylindrical or slightly swollen antheridium terminating a branch arising some distance below the oogonium from the same hypha as the latter, has not been observed.

The pathogenicity of the fungus to cabbage was established by inoculating healthy heads through incisions at the base of the stalk. Extensive water soaking in the region surrounding the inoculum became evident within 24 hours, the tissues of the core simultaneously becoming softened to a narrow-like consistency. Eight days after inoculation, on cutting the specimens, the condition shown in figure 1 was found: the core entirely softened, and the infection extending into all the foliar elements that make up the head for distances up to 60 millimeters. Although some mycelium was present in the spaces between the leaves, the fungus appeared to progress largely within the individual leaves, attaining its greatest development, as in the original specimen, in the tissues of the thickened fleshy

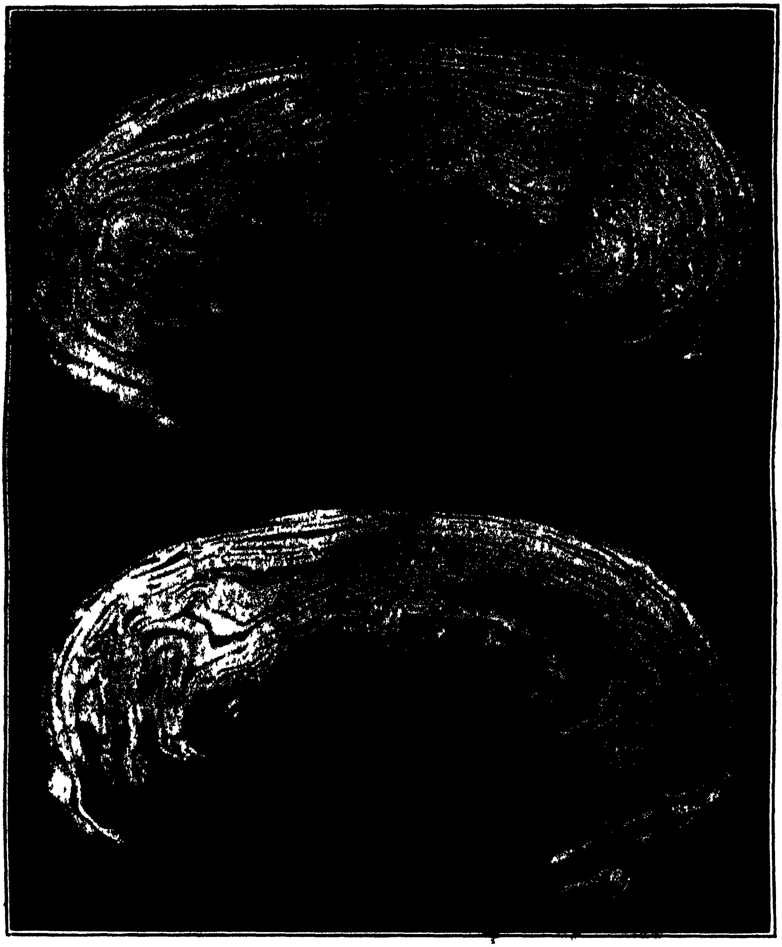

FIGURE 1. Two cabbage heads 8 days after inoculation at the base of the stump with a pure culture of the *Pythium* species isolated from a naturally infected head. Enlarged × ½.

midrib. Here a watery condition obtained not greatly unlike that which suggested the terms "leak" and "cottony leak" for diseases caused by congeneric forms in potatoes and cucumbers respectively, although, owing to preservation of the outer layers of tissue, the liquid is usually retained, so that the head, as a whole, does not become markedly wet. A mild, not unpleasant odor, as of stewing cabbage, appears to be produced by the activity of the parasite.

Concerning the prevalence of the disease in question nothing definite is known. The writer is informed that a type of damage quite similar to that resulting from artificial inoculations has been observed on cabbage in the New York market, where it is usually known among the trade as "stump-rot" and generally assumed to be a form of bacterial soft rot. It should be mentioned that as in other cases of Pythium infection, after the cabbage tissues have been killed, bacteria multiply, so that the parts take on the texture and emit the disagreeable odor of putrifying material. It is hoped that further observation may yield information concerning the prevalence of Pythium infection and its possible relation to stump-rot.

Inoculation experiments carried out on cabbage heads, using various species of *Pythium,* indicate, as might be expected from experience in parallel cases, that pathogenicity is not confined to the form isolated from the host in question. A number of species of the "debaryanum" type derived from various sources, as, for example, from potatoes affected with leak, from diseased pea roots, from diseased sweet-potato rootlets, from sweet-potato roots affected with mottle necrosis, and from watermelon fruit affected with blossom-end rot, produced similar pathological effects, in some instances more rapidly, in other instances less rapidly than the cabbage form. Strains of *P. aphanidermatum* (Edson) Fitz. isolated from diseased watermelon fruit and from cucumbers affected with cottony leak, also gave positive results. On the other hand, certain forms of the "debaryanum" type gave only negative results. None of the species with spiny oogonia appear to be capable of infecting cabbage, nor have any of the forms with a similar intramatrical habit but with smooth oogonia, among which *P. monospermum* Pringsheim could be definitely identified, shown any indication of pathogenicity.

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WASHINGTON, D. C.

FUSARIUM VASINFECTUM AND THE DAMPING OFF OF COTTON SEEDLINGS

H. R. ROSEN

During May, 1923, a very poor stand of cotton seedlings was noted in certain field plots in contrast to others in which the seed used had been the same and which showed a far superior stand. These plots had been artificially infested with pure cultures of the cotton-wilt fungus. In order to insure thorough infestation, the fungus growing on bran was heavily applied in open furrows, and the cotton seed was sown by hand directly upon the inoculated bran. Within a few days after planting and before the plants had appeared, a heavy fungus growth could be clearly seen across the field, marking the paths where the cultures had been applied. Considerable rain fell and there was very little sunshine during the period.

A field approximately one acre in extent had been used, and in cooperation with the Agronomy Department, University of Arkansas, the field had been divided into six sections and different fertilizers had been applied to one-half of each section. On three of these sections the unfertilized parts showed a very much poorer stand than upon the fertilized, and when seeds that had failed to produce plants were dug up it was found that some of them had apparently begun to sprout and had then been killed, while others had died apparently before the emergence of radicle or hypocotyl from the seed coats. A number appeared as seedlings just emerging from the hull, showing dark discolored and rotted parts of the hypocotyl, and without sufficient vigor to raise the plumule above ground. Also, of the seedlings that had pushed above the soil, quite a number showed typical symptoms of damping off and sore shin. From several such seedlings which had received surface sterilization, pure cultures of *Fusarium vasinfectum* were obtained, as ascertained by the color reaction on rice[1] and by inoculating sterilized soil with cultures and noting wilt development on plants grown in such soil.

It was of considerable interest to see that the stand was not equally superior on all fertilized, compared to unfertilized, plots. The 3 one-tenth acre plots receiving kainit (40 lbs.), muriate of potash (12 lbs.), and Utah potash (12 lbs.), respectively, showed greater stand, as compared to the unfertilized plots, than did any of the 3 following combinations: (1) acid phosphate (34 lbs.), nitrate of soda (12 lbs.), muriate of potash (4 lbs.); (2) acid phosphate (34 lbs.), nitrate of soda (12 lbs.), and muriate of potash (8 lbs.); and (3) acid phosphate (34 lbs.), nitrate of soda (12 lbs.), and Utah potash (8 lbs.). In all cases the fertilizer had been applied by hand in the furrows immediately on the bran cultures of the fungus.

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[1] Elliott, John A. Cotton wilt, a seed borne disease. Jour. Agr. Res. 23: 387–393. 1923.

There are two possible explanations for the greater stand on three of these fertilized plots: first, that the fertilizer having been in direct contact with the fungus may have considerably retarded its growth, or even killed it in those spots where the concentration of the dissolved salt would be considerable; second, that certain fertilizers exercise a stimulating effect on the germination of the seed or upon the growth of the seedling and enable it to withstand the attacks of this fungus.

As the relationship of the wilt-producing fungus to lack of stand, and particularly to damping off, was not considered clearly established in this field experiment, other tests were undertaken in the laboratory and in the greenhouse. (It is quite evident that under field conditions, with many uncontrolled and unknown factors, particularly soil floras, direct causes for lack of stand, etc., may be obscured.) Cotton seeds thoroughly delinted with concentrated sulphuric acid were planted in pots containing sterilized soil (pots of soil autoclaved for from two to two and a half hours at about 15 pounds pressure). As far as known, these seeds had come from healthy plants. They appeared sound and gave a high percentage of germination. The seeds having been thoroughly rinsed and dried after treatment with sulphuric acid were then soaked in a heavy spore suspension of *Fusarium vasinfectum.* Within two weeks after seeding 12 seedlings out of 24 which had shown dark discolored rotted tissue, usually around the soil line, had died of damping off; and in another pot, which had perhaps received greater amounts of water, most of the seed failed to germinate, or rather failed to appear above ground. Four died of damping off shortly after the cotyledons were free from the seed coats. In most cases the dead or dying plants were given a thorough microscopic examination, and in every instance the Fusarium fungus was identified. Numerous platings were made from attacked tissues, after the surfaces had been sterilized, and pure cultures of *Fusarium vasinfectum* obtained. On some plates, various bacteria were sometimes obtained along with Fusarium, but these were comparatively few. In no instance was Pythium or Rhizoctonia isolated, and the bacteria, mostly in the form of white colonies, were presumably saprophytes which had followed up the attacks of Fusarium. Likewise, the few deep yellow bacterial colonies which had developed around the plated tissue were very probably also saprophytes. *Pseudomonas malvacearum* Smith has often been cultured by the writer and its light yellow color on most media quite readily distinguishes it from yellowish saprophytes which may be present.

The above experiment was repeated with quite comparable results. Again seedlings with pronounced symptoms of collar rot and with Fusarium sporulating on the rotted part were surface sterilized and when plated out yielded cultures of only one fungus.

In still another series of tests, the sterilized pots were heavily inoculated with the wilt-producing fungus growing on cotton stems, and then seeded with acid delinted seed. Sore shin and damping off were again noted.

It is rather difficult to distinguish the damping off caused by Rhizoctonia from that caused by Fusarium. The writer has assumed, in common with other pathologists, that most of the damping off of cotton was due to Rhizoctonia, but, while this may be true, there is a strong possibility that *Fusarium vasinfectum* plays an important part in causing damping off, particularly in wilt-infested fields. There is not much difference in symptoms, as almost any kind of injury to growing cotton is likely to cause the reddish-brown or purplish coloring which is peculiar to cotton. However, in the case of damping off caused by Fusarium, the rotted part is usually dark in color, almost blackish, while that of Rhizoctonia is usually lighter—reddish wine-colored, or purplish. In addition to this, the darkened, rotted tissue, which is usually localized around the crown in this form of disease, has occasionally been found to extend upward through the xylem, beyond the rotted collar, comparable to the normal, darkened interior of wilted plants. Owing to the very rapid growth of Rhizoctonia on various nutrient media, particularly potato dextrose agar, it is very easy to obtain cultures of it if it is present in the diseased tissue. At about 28° C. it will outgrow most saprophytes, including bacteria as well as fungi. It is a much more vigorous grower than *F. vasinfectum* under conditions which are favorable to both.

Fusarium vasinfectum, like other vascular Fusaria, undoubtedly attacks through the roots and in common with these may be expected to do the most damage at rather high soil and air temperatures.[2] This would suggest that damping off, as well as wilting, can be expected when the weather is rather warm, whereas in the case of damping off caused by Rhizoctonia we may expect it to be worse during cool, damp weather.

Briefly summarizing—field observations, as well as laboratory and greenhouse tests involving pure culture inoculations, clearly indicate that *Fusarium vasinfectum* may prevent cotton seed from germinating properly and may attack and kill young seedlings in a manner quite comparable to damping off caused by other fungi and bacteria.

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[2] Jones, L. R. The relation of environment to disease in plants. Amer. Jour. Bot. 11: 601-609. 1924.

NOTES ON THE PARASITISM OF ENDOTHIA GYROSA (SCHW.) FR.

JAMES R. WEIR

WITH ONE FIGURE IN THE TEXT

During the summer of 1903, the writer began a series of inoculations with a number of forest fungi in a hardwood forest near Scottsburg, Indiana. The results obtained with *Endothia gyrosa* (Schw.) Fr. found on the roots of *Quercus velutina* are of interest since they corroborate the work of Shear, Stevens,[1] and Clinton,[2] but with a greater tendency toward parasitism.

The observation was made that wounds on the roots of black oaks (in particular, *Quercus velutina* Lam.) produced by the tramping of farm animals never heal when infected with the above fungus, but result eventually either in the death of the entire root or a part of it. Wounds not infected usually healed in a comparatively short time. These, among other observations, led to a supposition that this fungus was a slow parasite and hence to the beginning of experimental work.

Inoculations were made in healthy surface roots, inserting a wedge-shaped piece of bark and wood infected with the fungus into a corresponding cut in the healthy surface of a root. Before inoculation, the selected area as well as the piece to be inserted were washed in a solution of mercuric chloride and the wound afterward bound about with a cloth that had been steeped in a boiling mixture of tallow, beeswax, and rosin. This formed an impervious covering for the wound and was very durable. Ten different inoculations in all were made, five on *Quercus velutina* and five on *Fagus americana*. The first inoculations were all made in the fall of the year (1903) and were examined the following summer, and again in 1911.

Four inoculations on beech and three on oak were successful. The infections in beech by the following spring had extended outward from 2 to 3 inches from the wound in a very uniform manner. The bark and last annual ring only were attacked after the first eight months. The infection eventually progressed from 5 to 6 inches on either side of the original wound. By the spring of 1911 the infections had extended along the free

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[1] Shear, C. L., N. E. Stevens, and R. J. Tiller. *Endothia parasitica* and related species. U. S. Dept. Agr. Bul. 380. 1917.

[2] Clinton, G. P. Chestnut bark disease, *Endothia gyrosa* var. *parasitica* (Murr.) Clint. Conn. Agr. Exp. Sta. Ann. Rpt. 1911–12: 359–453. 1913.

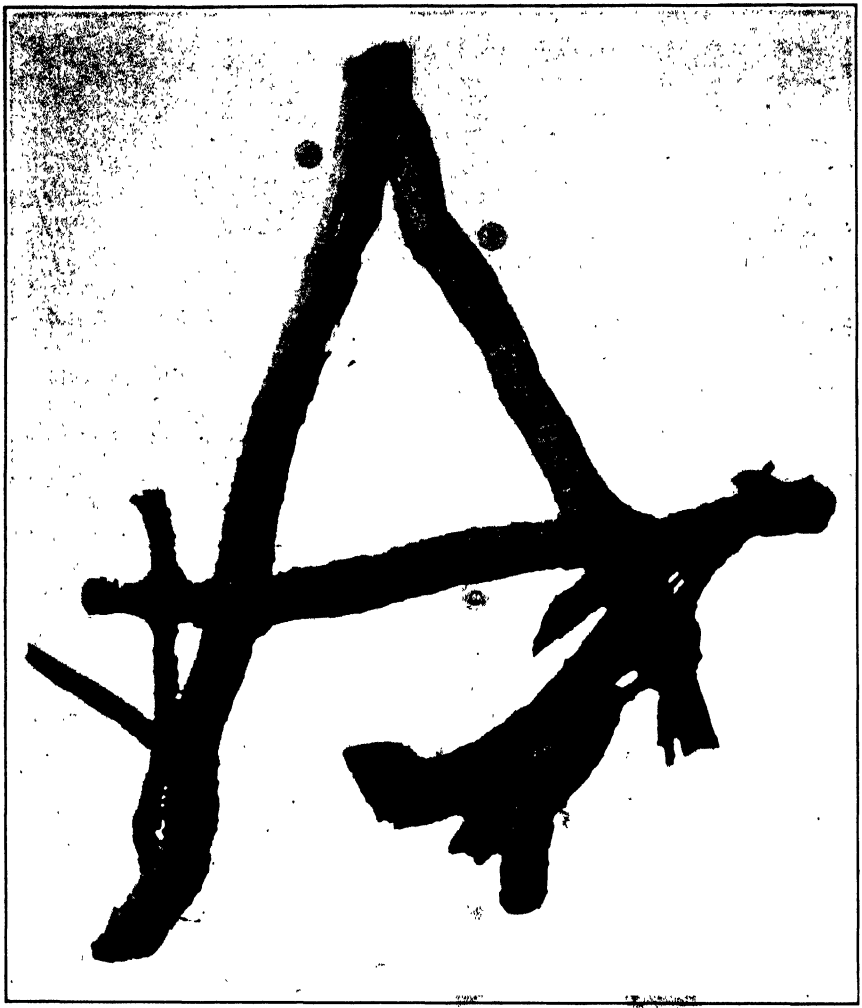

FIG. 1. Inoculation of *Endothia gyrosa* on living roots of *Quercus velutina*. A.—Point of inoculation. Infection spread to adjoining tree by means of grafted roots. Fungus visible at B and C.

upper surface of the roots of the oaks to the point where they became wholly covered with soil, an average distance of four feet. The fungus does not seem to have the ability of extending any appreciable distance along roots under the ground. The successful inoculations on the roots of the beech had extended but very little farther than those noted in 1903. In one case, where the root of the oak was small and free from the ground, girdling resulted, causing the free end of the root to succumb. In the other cases, only the free upper surfaces of the roots were killed. The yellow stromata made their appearance on either side of the cloth shield and gradually developed farther away from the original wound as the infections spread. In the spring of 1922 the inoculated roots were again examined. Of the four inoculations on beech, two infections had extended an average of one foot each way from the original point of inoculation. One had made but little progress and one had apparently died out. The infections were confined to this upper side of the roots. In the case of the infected oak, the fungus had become established in one instance on neighboring roots of another tree of the same species by means of grafted laterals (Fig. 1), the entire progress being an average of two feet. The progress of the remaining infections on oak was in like proportion, but was confined to the original root.

The inoculations were controlled by making exactly similar wounds in healthy roots and under exactly similar conditions. These wounds healed entirely with no apparent injury to the roots.

The fungus is by no means confined to the bark. The mycelium is fine, but by proper manipulation can be seen in the outer woody tissues, particularly in the larger cell elements. The mycelium in the woody portion of the root is confined principally to the last two or three annual rings. The chemical influence of the fungus extends much deeper and has a peculiar discoloring effect both on the bark and wood.

The fact that this fungus in artificially inoculated roots is able to gradually increase the infected area through the activities of its own mycelium, resulting in the death of the root or that part of it above ground, indicates the slow parasitism of this fungus. It enters entirely through wounds and will sometimes continue indefinitely producing its stromata on a very small infected area. This indicates the inability of the fungus to become parasitic during the early stages of growth. With a cumulative vegetative growth it may gradually acquire greater parasitic tendencies. The fungus may produce its stromata directly on a woody surface. They are usually, however, developed on the outer surface of the bark.

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NOTES ON SPOROPHORES OF POLYPORUS SCHWEINITZII FR. ON YELLOW PINE IN CALIFORNIA

Lake S. Gill

The heartwood of living yellow pine (*Pinus ponderosa*) is often affected with the brown carbonizing rot usually attributed to *Polyporus schweinitzii,* although in California positive identification is not generally possible owing to the fact that fruiting bodies are rarely found in conjunction with the decay. It is true that sporophores of heartwood-destroying fungi are far less plentiful in the semiarid climate prevailing in California pine forests than in the fog belt of the coast or in the Pacific Northwest. The fairly common occurrence of sporophores on Douglas fir in the same forest type, however, seems to indicate that the inhibition in yellow pine is governed by factors inherent in the host, rather than by climatic conditions.

The ample collections of the Office of Forest Pathology, at San Francisco, made in pathological reconnaissance and field studies on the California pine forests since 1910, contain only one sporophore of *P. schweinitzii* known with certainty to have been growing on living yellow pine. This had been found near Norfork, Madera County, at an elevation of about 2,600 feet. It was noted as occurring on the base of a living tree and, judging by some charred bark scales and resin adhering to the back of the specimen, had been issuing from a fire scar. Another sporophore of the fungus taken from a scar at the base of what was either a yellow or Jeffrey pine standing in the same locality mentioned above, and a third collected near Beckwith, Plumas County, at an elevation of 5,500 feet, from the inside of a yellow pine stump which had been hollowed out by fire, are the only other records of immediate interest in the collection. It may be significant that all three sporophores were connected with wounds.

Two additional cases of *P. schweinitzii* fruiting on living *Pinus ponderosa* in the pine forests of the Sierra Nevada were observed during the summer of 1924. In one instance sporophores of the stipitate type with a large round pileus, such as are commonly seen growing from surface roots of Douglas fir, were found at a distance of five feet from the base of a yellow pine 45 inches in diameter breast high. There were no fruiting bodies on the bole itself. When this tree was felled, a continuous column of decay was found to extend through the bole for over 35 feet, rendering the first two 16-foot saw logs commercially worthless and necessitating a deduction from the scale of the third. In general, cull due to *P. schweinitzii* is confined to the first log. The observation was made early in August near the Cow Creek Ranger Station, Tuolumne County, at an elevation of about 5,000 feet.

Some 30 miles south of this point, at an elevation of 5,400 feet along the road leading from Crane Flat into Yosemite Valley, a more unusual case was noted on May 30, on a yellow pine 40 inches in diameter at breast height. Not only were sporophores coming from surface roots, but 12 or 14 more were growing out from a healed fire scar which extended from the ground to a height of about 15 feet. These were most numerous in the lower part of the wound but were distributed at uneven intervals along its entire length. In size they varied from abortive knobs two or three centimeters in diameter to imbricate clumps of shelves 15 cm. high, 25 cm. wide, and 13 cm. deep. The irregular and convoluted character suggested impeded growth.

The evidence at hand lends further support to the presumption that in California the fungus is responsible for considerable decay in yellow pine. Apparently, however, it fruits sparingly on this species and produces sporophores on the bole only where an injury by fire or other agency permits the mycelium to grow out from the heartwood to the exterior of the tree. It is not impossible that the common form with the central stipe arising from surface roots also depends upon the presence of wounds for its development.

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PHYTOPATHOLOGICAL NOTES

Preliminary environmental studies on the take-all disease of wheat caused by Ophiobolus graminis sacc. The take-all disease has caused considerable damage to the wheat crop in foreign countries for many years and comparatively recently the disease has been found to occur in the United States. Although the take-all infested areas in this country are relatively small, it is important to note that as infection centers they are located at rather strategic points. The disease seems to have a firm hold in the center of the hard winter wheat belt, and observations show that it is steadily spreading.

Rather early in the investigations on take-all it was observed that the disease is more severe in certain districts than in others and also that in the same fields it is more pronounced some seasons than others. In connection with these observations on the development of the disease, irregularities in the fruiting habit of the parasite were also noted. Soil temperature and moisture are both important variables, and preliminary studies on the disease show that these factors influence its development to a considerable extent.

These studies were carried out in the greenhouse in Wisconsin soil temperature tanks. Owing to the cultural difficulties encountered when attempting to grow winter wheat under greenhouse conditions, it was not practicable to conduct the experiments over the entire life-history of the host, and accordingly all of the studies were confined to the early growth period. In all cases the experimental soil was inoculated with pure cultures of single spore strains of *Ophiobolus graminis.* Soil temperatures were maintained at intervals of approximately 4° C. between the extremes of 8° and 32° C. Soil moistures were varied simultaneously with temperature. They were based on the water-holding capacity of the soil and were maintained near 33, 54, and 71 per cent.

The results of four experiments show that under favorable soil conditions *Ophiobolus graminis* is an unusually vigorous root and tiller parasite. Although the fungus infected the host at all of the soil temperatures employed, the greatest injury occurred at unusually low soil temperatures (12°–16° C.). The injury was greatest at 12° C. in soil of the medium water content, whereas 16° C. produced the most severe injury in soils containing the least and the greatest amounts of moisture. Although the amount of injury was not great at 8° C., it was more severe than at 32° C. Diseased plants were rarely found in soil held near 32° C. At favorable soil temperatures infection occurred at all soil moistures, but the greatest amount of disease occurred in soils containing the highest percentages of water.

The results obtained with soil temperature are of interest when compared with those obtained in the similar studies on the Helminthosporium foot-rot. In the case of both parasites, the greatest vegetative development occurs near 24° C., whereas Helminthosporium foot-rot is greatest at soil temperatures near 28°–32° C., and take-all is most severe at 12°–16° C. In the studies on the Helminthosporium foot-rot,[1] the senior writer suggested that severe infection at the high temperatures might be explained by severe weakening of the host, due to unfavorable temperature conditions. Obviously this explanation will not hold for take-all, since under normal conditions thrifty, robust plants develop at 12°–16° C. It appears therefore that the explanations for these results must be sought through a study of factors probably relating both to the parasite and the host which are more basic than the phenomena of growth rate and vigor as revealed by ordinary methods of weight and measurement.

The details of these environmental studies will be published later.—H.H. MCKINNEY and R. J. DAVIS, Office of Cereal Investigations, U. S. Department of Agriculture, and Wisconsin Agricultural Experiment Station.

A Mosaic on Winter Wheat and Winter Rye. This disease on wheat has been reported previously by the writer and his collaborators in papers dealing with wheat rosette, but until recently the malady was not definitely known to be a transmissible mosaic. This disease seems to possess all of the symptoms associated with the mosaics of other grasses. It has been observed on wheat in the rosette-disease areas since 1920. In the spring of 1925, it was observed on winter rye growing in infested soil at Granite City, Illinois, and in the same soil which had been transported to Madison, Wisconsin, for experimental study. What appears to be the same mosaic was also found by Dr. A. G. Johnson in winter rye growing as a cover crop in the orchards of the U. S. Department of Agriculture, Arlington Farm, Virginia. Microscopic examinations of mosaiced rye plants from all these sources reveal the presence of cell inclusions which are very similar to, if not identical with, those associated with wheat mosaic.

Experiments have shown that the causal agent of this mosaic persists in the soil year after year. Susceptible varieties of winter wheat never have failed to develop the disease when grown in infested soil out of doors. By manipulating environmental conditions in the greenhouse, it has been possible to transmit this mosaic from diseased to healthy plants by means of the expressed juice and ground tissue. Five experiments have been carried out with Currell and Harvest Queen varieties of wheat. In every case the disease developed in some of the inoculated plants. A total of 160 Currell plants were inoculated and 17 of these developed mosaic and cell inclusions. One hundred and twenty-four Harvest Queen plants were inoculated. Twelve of these developed mosaic and cell inclusions.

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[1] Jour. Agr. Res. 26: 195–218. 1923.

It is of special interest to note that all but one of the Harvest Queen plants affected with mosaic became quite dwarfed. They also developed a deep green color. In fact, these plants presented an appearance which seemed identical with that of plants affected with wheat rosette. This condition has never been found to occur in the Currell variety. In no case did mosaic or the rosetted condition develop in the uninoculated plants used as controls, and no cell inclusions were found in any of the control plants examined.

While the percentage of inoculated plants which developed mosaic is rather small, this is not surprising as other grass mosaics have not been transmitted readily by means of the expressed plant juice. The fact that one mosaiced Harvest Queen plant did not develop the rosette condition may be explained on a genetic basis.

Observations made over several years show that some varieties of winter wheat which have been bred pure for agronomic and botanical characters may produce plants, when grown in uniformly infested soil, which differ widely in their susceptibility to dwarfing (rosette) and in their ability to produce certain types of mosaic mottling.

Preliminary results from head selection studies suggest that types of mosaic may be determined in part by inheritable factors which are not necessarily homozygous in a so-called pure line variety.

As yet there is no evidence indicating that wheat mosaic is seed borne.

The relationship between this mosaic and other mosaics has not been determined.—H. H. MCKINNEY, Bureau of Plant Industry, U. S. Department of Agriculture.

Aplanobacter insidiosum n. sp., the cause of an alfalfa disease. In a recent number of PHYTOPATHOLOGY there is a brief description by Dr. F. R. Jones[1] of a new vascular, bacterial disease of alfalfa. Since that note was written, Dr. Jones has proved even more conclusively that this disease, which is unlike any previously described on alfalfa, is caused by bacteria.

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[1] Jones, Fred Reuel. A new bacterial disease of alfalfa. PHYTOPATH. 15: 243–244. 1925.

The writer was asked to determine the cultural and morphological characters of the organism and the present note is preliminary to a joint paper now in preparation by Dr. Jones and the writer.

This new plant pathogene is briefly characterized as follows:

Aplanobacter insidiosum n. sp.

A short, non-motile rod,[2] measuring 0.7 - 1.0 × 0.4 - 0.5μ for single, and 1.8 - 2.0 × 0.4 - 0.5μ for paired rods. Capsules are formed on most media; no spores; aerobic; Gram positive; not acid fast.

Growth on culture media is very slow and never becomes heavy. Beef agar poured plates require 4–5 days and often longer for the colonies to reach a visible size. The growth is white at first, becoming pale yellow. Colonies on beef agar are circular; margins entire; surfaces smooth and shining; flat to slightly convex; usually uniform in structure and viscid in consistency. Media containing sugar produces a better growth than ordinary media, but in such media the yellow color of the growth is likely to become obscured partly or entirely by the formation in the growth of *blue* granules which impart blue, green, and violet colors to the bacterial growth. The medium is not stained. The growth on potato cylinders is also subject to this discoloration. Beef gelatin is slowly liquefied; Loeffler's blood serum is not liquefied. Nitrates are not reduced. No gas is formed. No indol or hydrogen sulphide is produced. Small amounts of acid are formed from dextrose, lactose, saccharose, and glycerine. There is a long delayed (16–20 days) curdling of milk. There is no growth in Cohn's solution. In Fermi's and in Uschinsky's solutions an occasional culture has clouded, but mostly there is no trace of growth. In spite of its slow growth the organism has considerable vitality and survives rather long exposures to sunlight, to freezing, and to desiccation.

The maximum temperature for growth is 31° C., the minimum temperature is below 1° C., and the optimum about 23° C. Its thermal death point is about 51–52° C. According to the descriptive chart of 1920 of the Society of American Bacteriologists, its group number is 5331–31135–1222.

The specific name, *insidiosum,* selected for this organism, is expressive of its rather slow but persistent progress in the host which is often seriously infected before there is any definite outward symptom of disease.—LUCIA MCCULLOCH, Bureau of Plant Industry, Washington, D. C.

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[2] No, or very doubtful, motility has been discovered, and the many attempts to demonstrate flagella have been unsuccessful.

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THE PROBLEM OF HOST SELECTION AND HOST SPECIALIZATION OF CERTAIN PLANT-INFESTING NEMAS AND ITS APPLICATION IN THE STUDY OF NEMIC PESTS[1]

G. STEINER

WITH EIGHT FIGURES IN THE TEXT

INTRODUCTION

One of the most fascinating chapters in the study of plant-parasitic nemas is that dealing with their ability to find and select the proper host plant. The whole complex of known facts and still open questions centering about this mutual relationship of plants and nemas is perhaps best designated as the problem of host selection. Although not so recognized, this problem has played an exceedingly important role both during the past and present in the many attempts towards an applicable control of nemic plant-pests. To prove this we need only to recall the catch- or trap-plant method of Kühn (28), the recently outlined "Reizpflanzen methode" of Baunacke (3), the crop-rotation methods, the method of activation of the cysts of the sugar beet nema by Rensch (45), etc. These are all based or built up on the known facts of the stimulative influence of the host plant upon the parasite. During the last twenty or thirty years much work has been expended on the study of the so-called susceptibility and resistance of certain plants, or plant varieties, to nemic pests with a view of the possible breeding of immune or resistant crops. All the endeavor along these lines did not lead to any notable result, because of the complete ignoring of the host-selection problem, which here is of fundamental importance. Most of the investigators noted the peculiar behavior of the pest-producing nemas in selecting their plant hosts, but unfortunately did not recognize the primary importance of these facts.

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[1] The writer is indebted to Dr. Hall, of the Bureau of Animal Industry, for some literature; to Dr. Godfrey, Mr. Leukel, and Mr. Thorne, of the Bureau of Plant Industry, for some nema material, and to Dr. Arzberger, also of this Bureau, for the unpublished results of his investigations on the morphology of the roots of so-called nema-resistant cowpea varieties. Dr. Cobb kindly revised the manuscript and made some helpful suggestions.

Besides these more practical questions, our host selection problem involves a series of important and interesting theoretical aspects: questions of physiology, morphology, genetics, soil chemistry, soil physics, etc., etc., are connected with it.

It is the aim of the present paper to outline the problem, to analyze its present state, to describe the whole host-selection mechanism combined with existing host specialization as seen in the light of our present knowledge, and to show its importance and application. The writer is convinced that his conception not only explains many facts brought up by the earlier and present investigators as inexplicable and contradictory, but also makes it possible to work out more effective methods for the control of nemic pests, as, for example, scientific crop-rotation methods. Furthermore it will give the breeders and searchers for resistant or immune varieties a sound basis for their experiments and will open many lines of further attack of the whole problem of nemic plant-pests.

GENERAL PART

The Behavior of Plant-Infesting Nemas in Selecting Their Hosts

Soil is the life medium of the nemas to be considered here, a medium which, compared with water and air, isolates its inhabitants to a high degree. Soil is not transparent and it opposes the greatest obstacles to any movement, and seems, therefore, to separate and isolate the members of its life association in a most pronounced way. Yet the soil nemas are perfectly able to orient themselves in this medium, and locate their food and also their mating partners, even at considerable distances.

Our chief species of plant-infesting nemas attack a large number of different plant species, which are called their hosts. But there are hosts of different degrees. By this it is not to be understood that some species are, in general, more preferred than others. Different populations of the same nema species may behave quite differently toward one host plant, that is, one population will attack it heavily, and another not at all. The different populations of a nema species may each have a different preferred host plant. If a number of different host plants are growing on a given soil area, the nemas in the soil will always find out and attack the one they like best, leaving the others unharmed, even though they too are favored host plants of that species of nemas. How they are capable of doing this is a problem of much interest and importance, and will be explained later, according to our present knowledge.

A number of examples gathered from literature best give an idea of the strange and peculiar behavior the nemas very often show in selecting their host plants. Heretofore no explanation was given for this behavior, although it greatly puzzled many investigators and was a source of much controversy and many mistakes.

A very significant case has been described by Liebscher (30) from the Agricultural Experiment Station at Göttingen, Germany. I tried to condense this case in the schematic figure 1. A plot of ground of the experi-

Plot planted for 13 years with peas and finally highly infested with a strain of H.schachtii specialized on peas	1 meter	Plot planted for 17 years with oats and finally highly infested with a strain of H.schachtii specialized on oats
●	Pisum sativum (and all its varieties)	○
	Vicia faba	○
	" sativa	○
	" cordata	○
	" monantha	○
	" hirsuta	○
	" villosa	○
	" zerratifolia	○
	" narbonensis	○
	Ervum lens	○
	Lathyrus cicera	○
	Cicer. arietinum	○
	Lupinus luteus	○
	" CruiKshansKii	○
	" albus Termis	○
	Soja hispida	○
○	Avena sativa	●
○	Hordeum sativum	●
○	Triticum "	●
○	Beta vulgaris	●
○	Brassica rapa and varieties	●
○	" Napus " "	●
○	" oteracea " "	●

● high infestation ○ no infestation ▨ partial infestation

FIG. 1. Schematic representation of the experiments of Liebscher with two populations of *Heterodera schachtii*, the one highly specialized on peas, the other on oats.

ment station was, with one exception, continually planted with peas for about thirteen years; finally it became so badly infested with *Heterodera schachtii* that the harvest amounted to only 4% of the seed material. Separated from this plot of peas by a path of one meter was a plot on which, for seventeen years, oats had been planted. This plot was also highly infested with *H. schachtii* and declared to be "oatsick." Liebscher then planted on both plots (separated only by the aforementioned path of one meter in width) a number of crop-plants in order to find out how the nemas would behave toward them. The schematic figure gives the results. The *H. schachtii* populations on these two plots behaved, as can be seen, very differently toward the same series of host plants. Plants attacked by the population which had lived for years on peas were not attacked at all by that which had lived continually on oats, and vice versa. It can easily be understood that Liebscher, through these experimental results, came to the conclusion that the pea-nemas were a new form (*Heterodera goettingiana*), because they did not attack the usual host of *H. schachtii*, the sugar beet. He was apparently wrong in this conclusion, but nobody can blame him in the face of his striking results.

Today we see the situation in Liebscher's case as follows: The *Heterodera* populations on both plots were probably first identical, starting from the same infestation. Then the population on the pea plot lived for years, that is, for a long series of generations, exclusively on peas, and became highly specialized on this host. The population of the other plot, however, for at least seventeen years, i.e., for about seventy generations, lived on oats and had become highly specialized on this plant.

The relations of *H. schachtii* to oats have also been discussed by Fuchs (19, pp. 946–947) who calls attention to the fact that sometimes oats are attacked as heavily as sugar beets, while in other cases they are only slightly affected. Fuchs mentions the possibility of the segregation of races but doubts whether this would explain all the observed facts. In general he found that the *H. schachtii* specimens which he used would attack sugar beets, white mustard, and oats, with equal facility. But on one occasion he was unable to get oats infested, whereas sugar beets and white mustard, sowed three weeks later between these same oats, were heavily attacked. Why this varying behavior? Apparently the last population of *H. schachtii* Fuchs worked with was, in a very pronounced way, specialized on sugar beets and their nearest related plant species.

Some investigators class the members of the Solanaceae as being nonsusceptible to *H. schachtii* (Hollrung (25) and Kühn (28) used potatoes as crop following catch-plant sowings), while later, various authors found that potatoes were heavily attacked and that some weeds from the Solanaceae family were the most important propagators (3, 65). These contradictory statements can easily be understood. The *H. schachtii* populations with which these different investigators worked were specialized along different host lines. For the same nema species, the chicory was hitherto regarded as one of the most pronounced non-susceptible plants, and yet Baunacke (3, p. 211) quotes G. Plümecke who found this very plant also attacked by *H. schachtii.* It seems quite as if a much larger number of plants than hitherto thought could be hosts of this nema if the latter has sufficient time to adapt itself to it.

In quite recent years a similar case of a population of *H. schachtii,* specialized to such a degree on peas that sugar beets were hardly affected by them, was recorded by M. Capus (10) from the Gironde in France. In connection with the statement, that, in the experiments of Kühn, the *H. schachtii* from sugar beets did not attack peas, Hollrung (24) mentions similar experiences.

Voigt (59, 60) recorded a *H. schachtii* population which had specialized on hops.

Barley was ordinarily considered to be non-susceptible to *H. schachtii,* and yet Märker (see Baunacke 3, p. 277) mentions the following case. On a field badly infested with this nema, barley was grown for three successive years. The first two years no noticeable injury occurred, but in the third year the crop was destroyed before harvest. This shows that an apparently specialized population may adapt itself in a rather short time to a new host plant and again become injurious.

A large number of similar observations could be added.

Very often the nemas of one and the same species, but from different plots or different localities (sometimes close by) or different countries, attack different crop-plants here and there, so that the same crop will be damaged in one place but left unharmed in another. Potatoes, for example, will be harmed in one field but not in another, even though the harmful nema is present in both places. As in Liebscher's case, the *H. schachtii* populations, in fields and plots which touch each other, will often behave very differently; in one plot they will attack certain weeds heavily, but will leave them unharmed in the neighboring field. Mr. Gerald Thorne mentioned such cases to me from the sugar-beet fields near Salt Lake City, Utah, where in one field *Chenopodium album* would be heavily infested but would be free from attack in the next field. In all these cases the crop-history of a field will probably give the key to an explanation.

It is of practical interest to mention here certain experiences with the trap- or catch-plant method of Kühn. The latter used as catch-plant *Brassica compestris* var. *annua* ("Sommerrübsen") for the sugar-beet nema; Fuchs (19), white mustard (*Sinapis alba*); yet Baunacke (3, p. 239) showed by experiment in Saxony and in Brandenburg that these same plants were never attacked by some populations of the sugar-beet nemas. In these two cases it could be proved that the nemas were highly specialized on sugar beets, for both populations apparently had lived exclusively on this crop for a great number of generations.

The foregoing examples show how differently various populations of a nema species will behave toward various plant species, all known to be its hosts. Some populations are rather polyphagous, others are monophagous.

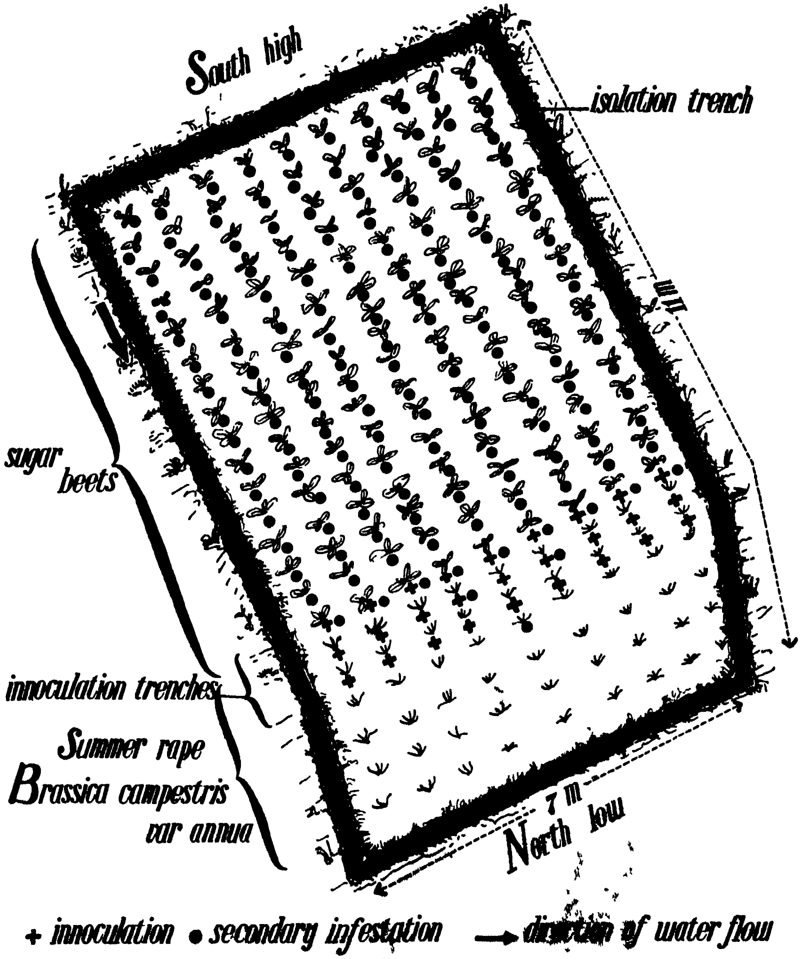

FIG. 2. Schematic representation of a field experiment of Baunacke to show the ability of *Heterodera schachtii* to locate its host plant. The population was apparently highly specialized on the sugar beet and, because it was left to choose, attacked only a few of the summer rape plants, although this is a good host plant and even used as a catch- or trap-plant. The nemas moved against the water flow. (The figure is made following the descriptions and two figures of Baunacke.)

The following example, one of Baunacke's experiments, will show a very astonishing faculty of the nemas, that is, their ability to locate, even at considerable distances, the preferred food-plant.

A plot 7x11m. (see fig. 2) hitherto absolutely free from *H. schachtii* was inoculated with a population of this species of nema, which for many generations had lived on sugar beets. The inoculation was made in November, 1919, in the following way. At the place marked on the schematic figure, three trenches fifty centimeters distant from each other, and thirty centimeters deep, were dug, and the bottom, about hand-high, was covered with soil containing *H. schachtii* and then filled again. To prevent emigration of the nemas, the plot was isolated by a trench of sufficient depth. In the next spring the 1.8m.-long northern part of the plot, including the inoculation trenches two and three, were planted with *Brassica campestris* var. *annua* ("Sommerrübsen"); the rest of the plot, including the inoculation trench one, with sugar beets. The plot inclined toward the north side, which is of importance in regard to the carrying faculty of the soil water which flowed in the direction indicated by the arrow.

As early as June 15th, the sugar beets in the inoculated trench one and in the immediate neighborhood were already infested, whereas the *Brassica campestris* var. *annua* plants in the inoculated trenches two and three were absolutely free. On September 28th, the nemas had already reached the southern end of the plot, whereas a careful examination of the *Brassica campestris* var. *annua* at the inoculation trenches showed only a few plants with a few females of *H. schachtii;* and, at a distance of one meter from the inoculation place, no infested *Brassica* could be found. Yet, as already remarked, the soil water moved toward the latter. This experiment, under free-land conditions, shows very well the ability of the nemas to locate their preferred host plants, even at considerable distances in the soil, and to move toward them in an active way, even against the water-flow.

A case which shows that the same ability to distinguish different plants exists in *Heterodera radicicola*[2] is given by Tischler (56, Figs. 3 and 4). This author found *Circaea lutetiana* and *C. intermedia* growing so closely together that, at the margin, the roots of both species intermingled. And yet he found *C. intermedia* absolutely free from *H. radicicola,* whereas the roots of *C. lutetiana* interminged with the former were infested. If, however, *C. intermedia* were planted in jars with soil from *C. lutetiana* containing the nemas, they would attack it, not having their preferred host at their disposal. This is a splendid example to show that the "botanical instinct" of our nemas enables them to distinguish even closely related plant species.

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[2] This form was recently placed in a separate new genus, *Caconema* (see 13: 118–119).

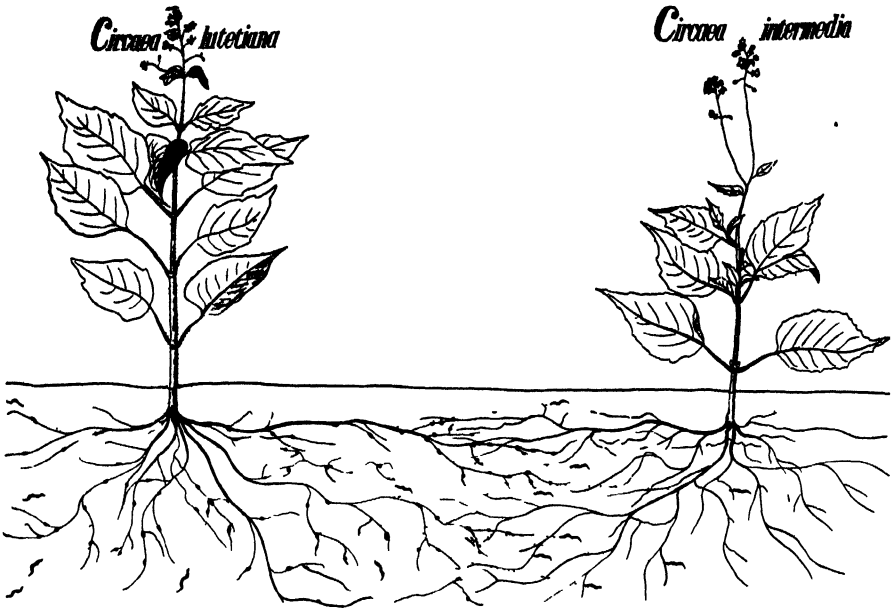

FIG. 3. Schematic representation of the behavior of a population of *Heterodera radicicola* as observed by Tischler. The nemas attacked only the roots of *Circaea lutetiana*, even though these roots were mixed with those of *C. intermedia*; the nemas were able to distinguish sharply both species.

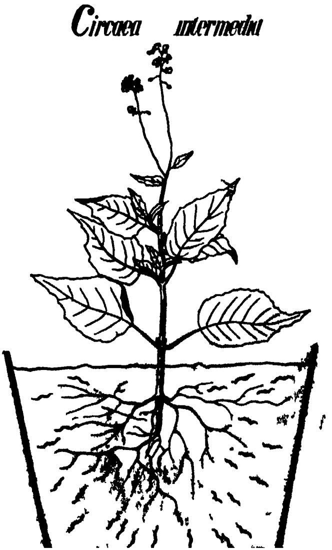

FIG. 4. Schematic representation of the behavior of the same population as in Fig. 3. Since the nemas had no other plant at their disposal, they attacked *Circaea intermedia*, which they had ignored under the previous conditions.

Atkinson (1) found *Amaranthus spinosus* at Auburn, Ala., not attacked by *H. radicicola* even in the neighborhood of badly diseased plants, whereas this same species is reported by Neal (37) as the "most dreaded and destructive agent in the spread of root-knot" in Florida.

But these faculties are possessed not only by the members of the genus *Heterodera;* they are apparently developed also in a similar way in many known plant-parasitic forms. Literature is especially rich in examples concerning *Tylenchus dipsaci,* which form, if taken as a whole, is very polyphagous, but which seems easily to develop populations more or less specialized on groups of plant species or even on single species. The bulb growers of Holland distinguish different strains specialized on certain plants, and Slogteren showed that these strains behaved in a way similar to that described above for *H. schachtii* and *H. radicicola.* As shown by figure 5, schematic sketches of Slogteren's (49) experiments, a *Tylenchus dipsaci* population which has lived on Hyacinths or on Narcissus, etc., will always attack this host first and leave the other plants unharmed. What astonishes us most here is the accuracy with which the nemas locate their preferred host plant.

These few cases are sufficient to show what we want; they could easily be multiplied.

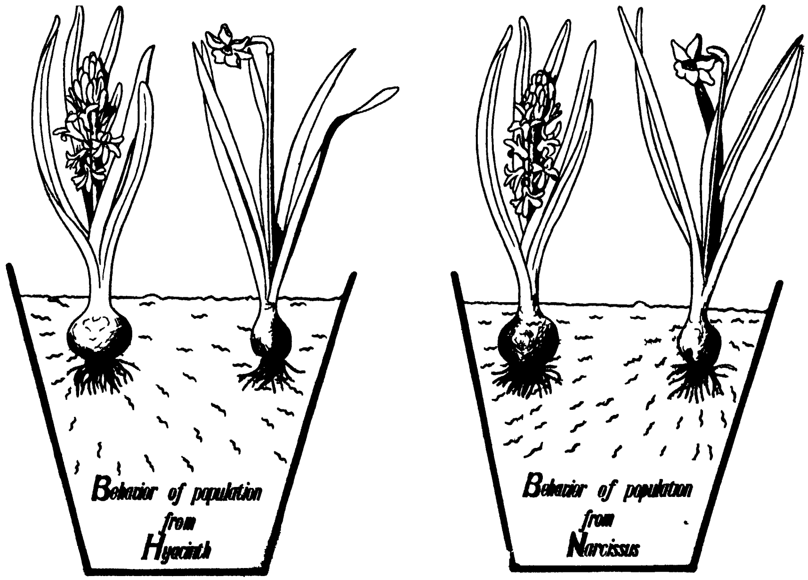
Behavior of population from Hyacinth
Behavior of population from Narcissus

FIG. 5. Schematic representation of the behavior of two different populations of *Tylenchus dipsaci,* as described by Slogteren. The one population lived on Hyacinths, the other on Narcissus. Therefore, if left to choose, the first population will ignore the Narcissus, the second the Hyacinth, for each will attack only the host of its ancestors.

The situation may best be characterized as follows: Although the different species of plant-parasitic nemas attack and feed on a wide range of host plants, a given population of one of these species will, if possible, always first attack the kind of host plant that its parents and ancestors lived on. If this plant is not available, in not being present or in being already highly parasitized, host plants of any kind of near relationship, taxonomical, chemical, etc., will be sought and attacked. If the ancestors of a population of one of the mentioned nema species lived during many generations on a number of different host plants, all these host plants and their related forms will be quite easily attacked. The population will then usually be found to be of somewhat polyphagous instinct. But, if the ancestors of a population have lived for a number of generations on a single species or even variety of host plant, their descendants may not attack any other plant known to be a host; or, if they do so, it will be only with difficulty, and then only in a small number. It might require generations before a new host is again attacked in the same degree as the old one. But a good part of the population apparently unable to accommodate itself to the new conditions starves to death, and in an extreme case only a few specimens, or even none, seem to have the ability to accustom themselves to the new host plant. Such nema populations are monophagous, and because of their specialization on one certain host plant were named "strains" or "biological or physiological races" in analogy to the well-known biological or physiological races or strains in some fungi, insects, etc. Most of the investigators accepted this conception and thought that they had in these nema populations forms with certain strongly inheritable characters. These "strains" played a prominent role in the experiments of all the investigators who aimed to breed so-called nema-resistant plant varieties. But unfortunately their true nature did not seem to be recognized. Nobody has been able, at least up to the present time, clearly to show morphological reasons for the establishment of definite strains, races, or varieties. Moreover, the experiments to reveal sharply cut physiological differences were very contradictory. One investigator could infest numerous plants with a "strain" from a certain plant, and a second investigator with a "strain" from the same plant but from other localities had no results. There are also many contradictions in regard to the host plants: one author states that a plant species is infested with a certain nema species, another one finds that it is not. But, in general, this strain, or biological race, theory was accepted. Only quite recently Baunacke denied the presence of any such races. This shows clearly that the former conception was not a satisfactory one and did not explain the multitude of facts.

In some papers on plant-infesting nemas, lists of highly susceptible crops and plants are often given with lists of others which are only slightly affected. From the foregoing study it results that such lists may have little absolute value. Apparently any plant said to be only slightly affected by a nema pest may become highly affected if the nema has time to adapt itself and to specialize on this particular host.

Since the behavior of a given nema population toward a host plant is largely dependent upon the nature of hosts on which the ancestors of the nemas lived, many mistakes were made in experiments during the past, especially in those on host resistance, host immunity, and in the so-called susceptibility tests. That something was wrong in the fundamental conceptions of all these experiments could easily be seen from the contradictory results obtained. Observations of one investigator were very often denied by another, as shown below. From the previous section, it can be concluded that any resistance, immunity, and susceptibility experiments with a given nema population may easily be incomplete, misleading, and unscientific, unless the host history of this nema population is known. A few examples from literature will show this.

Ramsbottom (43, p. 54) wanted to test the statement of Ritzema Bos (47, p. 308) that the Narcissus "strain" would not affect onions, but he failed, for the specimens that he took from Narcissus happened to infest onions badly. Why was this so? Ramsbottom unfortunately does not tell us how long his Narcissus "strain" had lived on Narcissus and whether a few generations ago it had been in contact with onions. The knowledge of the hosts for at least a number of immediate ancestral generations, however, seems to me exceedingly desirable, if not indispensable.

He succeeded also in infesting *Scilla nutans* with the same Narcissus "strain," which perhaps is an additional point to sustain our conception that he did not work with a highly specialized Narcissus "strain." Ramsbottom adds to these rather critical statements of the biological-strain theory, however, that on a visit to Spalding he made some contradictory observations. In two instances onions, lucerne, and clover (all susceptible crops) were found growing in close proximity to diseased Narcissus bulbs, but no damage appeared to have been inflicted to the former crop.

We are quite convinced that if Ramsbottom had exposed simultaneously to his Narcissus population Narcissus, onions, and *Scilla nutans,* he would have obtained only infested Narcissus. In the case of his field observations the Narcissus "strain" could choose, and therefore attacked, only Narcissus, and left the onions, lucerne, and clover unharmed.

Perhaps his experiments do not disprove the theory of "biological strains," but possibly if he had had a Narcissus strain which for long generations had lived exclusively on Narcissus, he would have obtained only a low degree of infestation in the onions and *Scilla nutans* that he chose, or even one not at all perceptible.

It is one thing to put an animal in a position to choose its food, but it is quite a different thing to force it to take a given food in order to escape starvation.

Very significant also are some experiments carried out and described by Goodey (22). This author wanted to test the susceptibility of certain crops and legumes for *Tylenchus dipsaci.* He took the nemas from an infected red clover field and gives the folowing table as expressing the "intensity of susceptibility" of the different plants:

Red clover (Canadian)	316	Group 1
" " (French)	215	
" " (English)	190	
Cow grass (Swedish)	167	
Kidney vetch	163.75	
Red clover (Wild English)	160	
Cow grass (English)	38.25	Group 2
Alsike clover (Canadian)	37	
" " (English)	28.5	
Sainfoin	7	Group 3
White clover (Wild Cotswold)	5.5	
" " (English)	4.75	
" " (Wild Kentish)	2	
White clover (Sutton's Mammoth)		Group 4
Lucerne		
Trefoil		

Therefore Goodey concludes that the susceptibility of group 1 is very high, that of group 2 much less, that of group 3 only slight; and group 4 appears to be non-susceptible. But let us quote the final statement of Goodey himself: "The results have a practical bearing of considerable importance to the farmer whose land is infested with *Tylenchus dipsaci,* and whose red clover is therefore liable to attack from this parasite. If he wishes to avoid stem disease, he should not sow red clover, cow grass, or alsike clover, but should make use of trefoil, lucerne, sainfoin, or a large white clover, such as Sutton's Mammoth White."

Yet lucerne (alfalfa) sometimes is one of the crops most badly affected by *T. dipsaci.* How can this be true considering Goodey's results? The nemas used for his experiment were taken from a red-clover field and apparently he had a population of *T. dipsaci* rather highly specialized on red clover, which very well explains the results of his susceptibility tests in the light of our conception. His conclusions would be all right if he would make the reservation that they are for such a specialized population of *T.*

dipsaci, but Goodey is certainly too hasty in generalizing such conclusions for *T. dipsaci* as a whole. We are convinced that this red-clover population of the present nema would soon have adapted itself to lucerne and other host plants. To recommend in this case host plants not susceptible to the specialized red-clover population, and of annual nature would have been perfectly safe, but certainly not lucerne and other hosts of perennial nature. This perennial nature would be the best opportunity for the nema to adapt itself, and perhaps in two or three years lucerne (alfalfa) would be highly infested.

The previous example shows well how the advocated conception explains observations and facts of hitherto rather puzzling nature, and gives more definite lines along which to work in crop rotation.

These examples may be sufficient to show one reason why so much of the endeavor of a series of investigators to find and to breed nema-resistant crop varieties had not yet produced the desired results, even after about thirty years of experimenting. The fundamental conceptions upon which these experiments were founded were inadequate. The terms "strain" and "biological race" were also often used in a very inexact and unscientific way. It is not allowable, for instance, to call a population of *T. dipsaci* a "strawberry strain" just because these nemas were found on strawberries. Before doing this it should be ascertained how long this population had really lived on strawberries and to what degree they were specialized on them. It is very incorrect so to name it if the specimens of the population also attack alfalfa, red clover, potatoes, buckwheat, peas, onions, etc. It seems to me that only a population which for many generations has lived exclusively on this plant and is therefore specialized on it should be so termed. But if the host history is not known, and experiments do not prove a strong specialization on strawberries, the term "strawberry strain" is certainly misused. A strain is, indeed, something more stable, at least as the term is used in genetics. It is of no value to state that the "alfalfa strain" of *T. dipsaci* can be brought to infest red clover, buckwheat, alsike, white clover, peas, turnips, potatoes, and Polygonum, if the former host history of this "alfalfa strain" is not known. In this host history of the "alfalfa strain" lies the explanation of its present behavior, and from a knowledge of this host history, one might even be able to predict behavior toward new host plants. Such "alfalfa strains" of different origin and unknown host history might behave quite differently and the results of such experiments would lead only to confusion. The behavior of these "strains" seems to be very largely a function of their host history—of the time they lived on a certain plant; and, as experience proves, it is therefore something very variable.

The conclusion therefore is that all experiments on nema resistance, immunity, and susceptibility of plants should be made only with populations of which the host history is well known for at least a number of generations. It is very doubtful whether a nema population of *Heterodera radicicola,* for instance, could be found, of which the present members could be brought to attack all the different plant species known to be its hosts.

However, it would seem possible to attract the offspring of a nema population of *H. radicicola,* for example, to all the known host plants by changing the host for the succeeding generations so that each following host would be of the closest possible relationship to the foregoing one, and to try in succession all the known hosts. Perhaps it would be possible in following this same line to infest a large number of plants not yet known as hosts.

Another point of practical use results from the preceding deductions, namely, that any crop variety should be claimed to be nema-resistant or immune or non-susceptibile only after this crop has been brought into contact, for at least a number of years, with a nema population which has lived in large numbers on a crop variety closely related to the one being tested. As we understand the situation, a nema population may adapt itself to a new host little by little, and only after some time be truly specialized on it. No statement that a crop variety is nema-resistant can be fully accepted until the host history of the nema population with which the experiments were made is properly known and stated.

The application of the present findings may be of some importance in crop rotation also. Usually it is said that any so-called nema-susceptible crop should be left out of the crop rotation on infested fields. However, as results from the previous sections show, it might in many cases be perfectly safe to use annual crops known to be hosts of the infesting nema species, as, for example, in the case described by Liebscher (30) and mentioned above, it would have been perfectly safe to grow sugar beets on the plot where *Heterodera schachtii* has damaged the peas so badly. A summer's crop of sugar beets would not have suffered from that *H. schachtii* population which had lived on peas for so long a time. On the plot where oats were so badly infested with *H. schachtii,* peas would have been safe.

For combatting nemic pests by scientific crop rotation, one has to know the host history of the nema population in a field (not only the crop raised but also the weeds present every year) and, armed with this knowledge, one may use even crops susceptible to the species of nemas in the field, but not to the present population.

HOW ARE THE NEMAS ABLE TO RECOGNIZE AND SELECT THEIR HOST PLANTS

The facts revealed in the foregoing show clearly that our plant-infesting nemas have the faculty of recognizing a host plant and even of distinguishing a preferred host plant. The preferred host plant seems always to be that one upon which the ancestors of the nema population lived. The degree of preference for a certain host plant seems to grow with the number of successive generations a population of a nema species has lived on it; and the more the nema specializes on one host plant, the more it will apparently lose the ability to attack other host species, and the more finely developed will its sensibility for this one preferred host become (3). Now the question arises as to the causes of this significant behavior. Are they within the plant, the nema, or within both?

Baunacke (3) showed that the nema is led to its preferred host plant by some chemotactic influence exerted by the plant. His experiments seem to prove that some kind of root secretions, chiefly formed by young growing roots, are carried by the soil water to the nemas, which, stimulated by this substance, even leave the egg shell or the cysts, a fact recently developed further by Rensch (45) in some very interesting and promising experiments. Moving towards the higher concentration of the dissolved substance they find their host. It has to be assumed from these experiments that the active root secretions of different plants must be different and that the nemas are capable of recognizing the differences and of choosing those of the preferred host plant first.

However, taking into account the more recent experiments of Rensch, it seems at first that the active part of these root secretions is of a rather simple chemical nature. Rensch confirmed the results of Baunacke that washings of the roots of growing sugar beets, containing the active parts of the root secretions in solution, would stimulate the larvae contained in the brown cysts of *Heterodera schachtii* to hatch. Rensch was even able to get the same results by the application of two synthetical products, named A and B, prepared by a chemical firm. What he says about their chemical nature is that the first, A, is a known stuff from roots of many plants and that the second, B, is formed by the transformation of plant plasma in the soil. It therefore seems that these active compounds must be of a rather simple chemical nature. But, if we compare these results with all the facts of the ability of the nemas to respond to and to distinguish the stimuli of different plant species and even varieties, it seems impossible that these compounds are the only directing stimuli. From the results of the experiments of Rensch and a study of the behavior of plant-parasitic nemas toward their hosts, I rather conclude that the compounds which stimulate the nemas to hatch are not identical with the ones which direct them afterwards to their preferred host plant. Compounds generally formed by the growing of roots and of little or no specific character might act like first messengers on the encysted larvae of the nema and announce to them that conditions for leaving the cysts were good. Naturally the larvae will move toward these stimuli, because they are signs of plant life, but the final selection of the host seems to me to be directed by compounds of more specific nature.

This conception seems also to be supported by the action of chicories upon the sugar-beet nema. Several authors proved that these plants stimulate to a high degree the hatching of the larvae in the brown cysts, but no nemas or very few will attack it.

In showing the chemotactic influence of the plant upon *Heterodera schachtii*, Baunacke, and later Rensch, made a most important step toward an understanding of the mutual relationship of host and parasite. Without doubt similar conditions are present in *H. radicicola*, *Tylenchus dipsaci*, and other plant-infesting nemas and their hosts. The nemas must have means to recognize and distinguish their hosts through chemicals secreted by the latter and carried by the soil water. The nature of the active root secretions is not yet known, but must doubtless be very specific for each plant species, sometimes even for plant varieties or races. Neither Baunacke nor Rensch traced these relations any farther. The nema, as the perceiving organism, was not studied more closely by them; they seemed to be satisfied with the establishment of the relationship. Yet, for a true understanding, we need to know how the nema is able to perceive the chemical stimuli sent out by the plants. Has the nema organs of perception for such stimuli? Undoubtedly these organs can only be sense organs. Furthermore, they must be placed near or at the head end. If not, they could never guide the nema toward its host. The head end of the nemas, in fact, shows at least two, sometimes three, groups of sense organs differing decidedly in structure and undoubtedly also in their functions. These are, first, the so-called head, labial, and mouth papillae, which are thought to be organs of touch (tangoreceptores); second, the amphids, or lateral organs, which by zur Strassen (55) and the writer (50, 54) have been claimed to function as chemical sense organs (organs of taste, gustoreceptores); and third, in some marine and fresh-water free-living forms there are also organs for the perception of light (ocelles, photoreceptores).

The amphids, or lateral organs, are, as the writer thinks, doubtless the sense organs with which the nemas perceive the chemical stimuli which Baunacke and Rensch in their experiments proved to be sent out from the roots of sugar beets.

These amphids are well known to all investigators who have worked on free-living nemas. They were first named lateral organs by Bastian (2), because of their constant lateral position. Only exceptionally are they shifted slightly dorsal. They were later renamed amphids by Cobb (12) because animal morphology had already lateral organs in fishes, amphibians, etc.; also, and more important philologically, the nemas have many other organs that are "lateral." There is no doubt that they are not homologous with the former, and it was therefore thought best to rename them. Until quite recently amphids were assumed to be a feature of only some free-living genera. Investigations, chiefly by Cobb and the writer, showed that they are present also in many parasitic forms from widely separated groups. As they were found in any nema which was closely searched, it might be safe to say that they are present in all nemas, and are their most characteristic and typical feature. Textbooks could safely assume this character to be typical in their diagnostic descriptions of nemas, if once they would try to become modern in this respect and not offer again and again chapters on nemas written exclusively about a few parasitic forms, neglecting the bulk of the group in all its morphological and physiological richness.

Years ago zur Strassen (55) pointed out that the so-called lateral papilla, as described by authors of parasitic nema papers, might be homologous to the amphids. Again and again investigators showed that these so-called lateral papillae were entirely different in structure from the submedial ones, and the presence in them of apparently glandular cells connected with nervous elements was repeatedly stated.

Recent investigations of Cobb (13) showed the presence of these amphids in *Heterodera radicicola,* and the present study attempts to describe them also for *H. schachtii, Tylenchus dipsaci,* and *T. tritici.*

It may be repeated here that Baunacke was not the first who observed such pronounced chemotaxism in nemas; similar observations were made by a number of other investigators such as Buerkel (9), Marcinowski (33), etc. Having adopted the views of zur Strassen about the assumed chemical function of the amphids, the writer himself has called attention to this in many of his previous papers (50, 51, 52, 53, 54).

It is of special interest to know that these organs are also present in *Heterodera schachtii,* because it is for this form that the chemotaxic relationship between plant and nema has been proved experimentally by Baunacke. Here the amphids have the usual lateral position, and are somewhat difficult to see; this is the chief reason why they have been overlooked in the past. The amphidial opening is very close to the mouth opening (Fig. 6C) and has, as seen from the front, a somewhat oval shape. A narrow canal leads into a long conical structure which can be followed for a distance towards the nerve ring. Just behind the spherical swellings on the inner end of the spear, the cavity of the organ appears to be closed, so that the cephalic part seems to form a pouch-like structure, inside of which a small number of threadlike fibers can be seen. These are apparently terminals of a bundle of nerve fibers. The cross-sections represented in figure 6 (D and E), combined with figure 6 (A and B), enable one to get a conception of the organ more or less approaching the reality. The writer is inclined to assume that, as in other nemas, a cell of apparently glandular nature surrounds the nerve fibers, connecting the terminals with the central nervous system, and that this cell begins right where the amphidial pouch is closed.

Cobb (13), as mentioned above, recently described a very similar structure for *Heterodera radicicola*.

Also *Tylenchus dipsaci* proved to have these amphids, as shown in figure 7 (A–D). A front view is given in figure 7C, and the openings of the am-

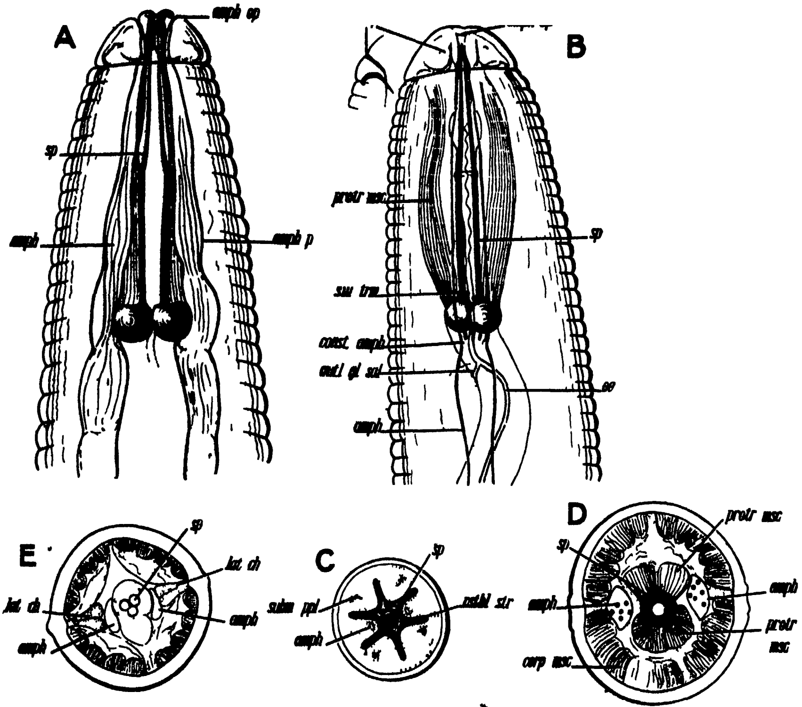

FIG. 6, A *Heterodera schachtii*, head end, seen from a medial side, to show the amphid on each lateral side; *amph*, amphid; *amph op*, amphidial opening; *amph p*, amphidial pouch; *sp*, spear.

B. *Main fig. Heterodera schachtii*, head end, seen laterally; *left small fig*, sketch of a submedial head papilla as seen in profile view; *amph*, amphid; *amph op*, amphidial opening; *const amph*, constriction of the amphid, apparently the place where the amphidial pouch ends, *oe*, oesophagus; *outl gl sal*, outlet of the salivary gland; *protr mso*, protractor muscle of the spear; *sp*, spear; *subm ppl*, submedial papillae; *sw trm*, probably a swelling in the terminal.

C. *Heterodera schachtii*, front view of the head; *amph*, amphid; *sp*, spear; *subm ppl*, submedial papillae; *vstbl str*, vestibular structure.

D. *Heterodera schachtii*, cross section through the head in the region of the cylindrical part of the spear; *amph*, amphid; *corp msc*, body muscles; *protr msc*, protractor muscles of the spear; *sp*, spear.

E. *Heterodera schachtii*, cross section right behind the bulbs of the spear; *amph*, amphid; *lat ch*, lateral chord; *sp*, spear.

phids are easily distinguished from the four submedial papillae. Other aspects of the organ are shown in figure 7A; a somewhat sublateral view, figure 7B; a medial view showing the organs in profile in figure 7D, where a cross section is drawn. In the specimens of this species, swellings of the terminals, as described by Cobb for *H. radicicola,* could be seen, and the whole structure of the organ was even better recognizable than in the foregoing *H. schachtii.* These are, however, apparently results of the fact that the specimens of *T. dipsaci* which were used for the present study were adults, whereas those of *H. schachtii* were still in the young larval stage, therefore much smaller and more difficult for the study of details.

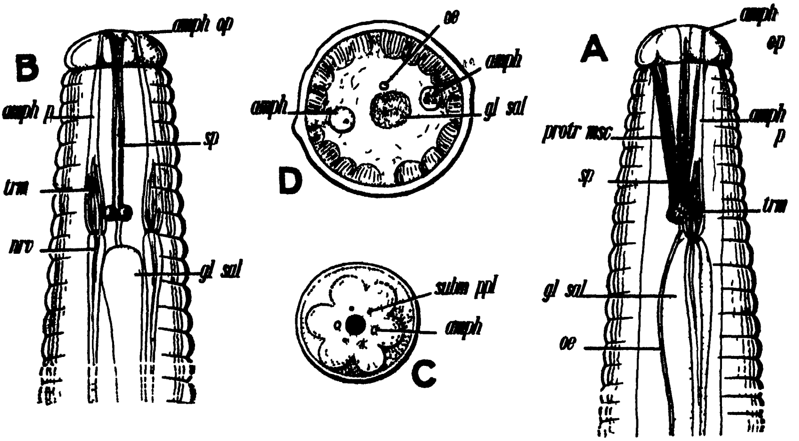

FIG. 7, A. *Tylenchus dipsaci,* ventro-submedial view of head end; *amph op,* amphidial opening; *amph p,* amphidial pouch; *oe,* oesophagus; *protr msc,* protractor muscle of the spear; *sal gl,* salivary gland; *sp,* spear.

B. *Tylenchus dipsaci,* ventral view of head end; *amph op,* amphidial opening; *amph p,* amphidial pouch; *nrv,* apparently amphidial nerve fibers; *sal gl,* salivary gland; *sp,* spear; *trm,* terminals.

C. *Tylenchus dipsaci,* front view of the head; *amph,* amphid; *subm ppl,* submedial papilla.

D. *Tylenchus dipsaci,* cross section through the region behind the spear; *amph,* amphid; *gl sal,* salivary gland; *oe,* oesophagus.

In *Tylenchus tritici* the amphids are very similar to those of *T. dipsaci* and of the two species of *Heterodera.* A front view shows two circles of head sense organs; the inner circle comprises very fine and minute mouth papillae. There are apparently six faintly developed lips (Fig. 8A), and the lip region is somewhat set off. The submedial lips bear each a very fine cephalic papilla, more easily seen in a front view. The lateral lips, however, seem to be without any papillae but to bear the amphids. In a front view they closely resemble the submedial papillae, but intra-vitam staining shows the difference in structure very quickly. The amphidial openings are apparently placed on the apex of the lateral lips, and are therefore located in the same circle with the submedial papillae. A very narrow duct leads inward and gradually widens to what might be termed a long, conical, or better, a somewhat irregularly shaped, spindle-like, amphidial pouch with thin walls. This pouch seems to end in about the double length of the spear behind the head end, as indicated by a constriction, after which a more compact part follows. Within the pouch the same elements as in both species of *Heterodera* and *T. dipsaci* could be seen, but it is very difficult to ascertain the number, exact shape, and position The cross sections as shown in figure 8 (C and D), combined with the views of figure 8 (A and B), may help to present the structures in the best possible way.

If we look for organs of similar structure throughout the animal kingdom, so-called chemical sense organs (taste and smell) of other forms are the first to be considered, as, for example, the taste buds of mammals or insects, etc.

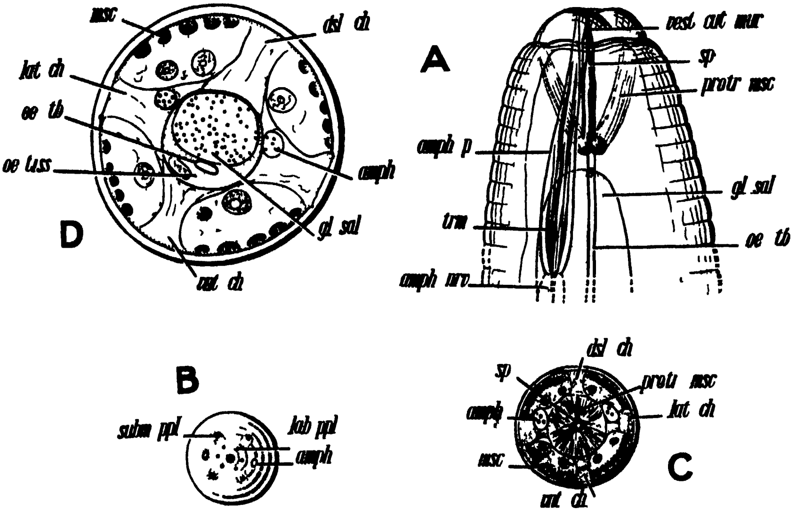

FIG. 8, A. *Tylenchus tritici*, dorso sublateral view of the head end; *amph nrv*, amphidial nerve; *amph p*, amphidial pouch; *gl sal*, salivary gland; *oe tb*, aesophageal tube; *protr msc*, protractor muscle; *sp*, spear; *trm*, terminal; *vest cut mur*, cuticularized wall of vestibulum.

B. *Tylenchus tritici*, front view of head end; *amph*, amphid; *lab ppl*, labial papillae; *subm ppl*, submedial papillae.

C. *Tylenchus tritici*, cross section through the region of the spear; *amph*, amphid; *dsl ch*, dorsal chord; *lat ch*, lateral chord; *msc*, body muscles; *protr msc*, protractor muscles of spear; *sp*, spear; *vnt ch*, ventral chord.

D. *Tylenchus tritici*, cross section through region behind the spear; *amph*, amphid; *dsl ch*, dorsal chord; *gl sal*, salivary gland; *lat ch*, lateral chord; *msc*, body muscles; *oe tb*, oesophageal tube; *oe tiss*, oesophageal tissue; *vnt ch*, ventral chord.

We have not yet established the connection of the above-described end apparatus of the amphids with the central nervous system. It will be done later; but as this connection has been studied and found to exist in a number of nemas from widely separated groups, and to be of similar structure throughout, the acceptance that the present forms make no exception is certainly permissible. In all probability our parasites do not differ in this point from the typical organization found to be valid for Ascaris (21), Oxyuris (34), Sclerostomidae (31a), Ancylostoma (31), Siphonolaimus (55), Oncholaimus (18), Mermithids (23), etc.

It might be said that we have not yet the experimental proof of the chemical function of the amphids or lateral organs, especially in the present species. This proof will be a very difficult one technically on account of the small size of our parasites and the much smaller size of the organs themselves. To make these organs functionless, and then to study the behavior of an animal deprived of them will be a difficult proposition. Perhaps some free-living marine forms with large-sized amphids could best be used for such experiments. But while experimental proof of the nature of the function of the amphids of nemas is still lacking, there are a number of reasons which speak plainly for their chemical function.

1. Their structure is most often very similar to that of the chemical sense organs of other animals (organs of taste and smell), especially to the taste buds.

2. The amphids show, within the nematode group, a multitude of different forms for which only the conception of their chemical function seems to give a satisfying explanation. In assuming for them a statical or auditive function, however, this structural richness can not be understood; gravity can not be thought to affect differently the various species of one genus or the varieties of one species. And yet the amphids often show marked structural differences even in very close systematic groups. Neither can the conception of their auditive function be satisfactory in view of the apparent specificity of the amphids in each variety, species, and genus. Even if we assume that the environment of nemas is filled with sounds, we are unable to see a relation of specific environmental sounds to the nemas; we can not see any benefit to the nema in perceiving these sounds, and nature never works along lines of uselessness. Neither can any other kind of mechanical waves or pressure explain in a satisfactory way the structural richness of the amphidial organs in our nemas.

3. Numerous cases of so-called sexual dimorphism are recorded in the amphids of nemas, the ones of the males very often being larger than those of the females. Only the conception of their chemical function furnishes a satisfying reason. If they were auditive in their function such sexual dimorphism could only be understood by the conception that the sexes produce

tunes to attract each other and that these tunes are specific for even varieties of species. But no tune-producing organs are known in our nemas. Nor can the assumption of a statical function or a mechanical wave-perceiving function explain this dimorphism.

4. The amphids are probably, without exception, a compound organ of nervous elements with what seems to be a gland. Here, too, the conception of a chemical function seems the one most in accord with this union of organ elements.

5. That the nemas must have chemical sense organs is experimentally proved. If we review all the known sense organs of the nema body, the amphids are, it seems to me, the only ones which could be considered as such, as the structure or position of all others is of a nature which contradicts their identification as chemical sense organs.

6. It is a rule that the amphids of nemas living free in fresh water and sea water are most often of a comparatively larger size (e.g., the end apparatus) than those of the parasites and of the free-living soil forms. This can easily be understood from the conception that the amphids are chemical sense organs and that in the sea and lakes naturally the active chemicals are dissolved more than, for example, in intestinal fluids or in the soil water. The larger size of these organs in fresh-water and sea nemas can easily be explained by this conception.

Thus it seems that many facts speak for this conception, and, so far as I know, none against it.

With the foregoing facts and interpretations which have just been set forth, the whole mechanism of host selection of the plant-parasitic nemas is satisfactorily outlined in its chief points and may be described as follows: Apparently some very specific chemical substances are produced by the growing roots of plants, and are dissolved and carried by the soil water in various directions. They act as stimuli on the nemas, the latter having receptors for them, presumably the so-called amphids or lateral organs on the head end. By moving toward points of higher concentration of the solution, e.g., towards quantitatively higher stimuli, the nemas locate their hosts. The amphids as perceiving organs are highly sensible to the qualities as well as quantities of the acting chemicals.

THE HOST SELECTION OF NEMAS PARASITIC IN ANIMALS

The question arises whether the host selection in nemas parasitic in animals is similar to that here described for plant-parasitic forms. Undoubtedly this might be so in many cases, at least in those where the parasites actively move toward the host. It might not be so in all cases where infection goes on in a more or less passive way and is built upon coincidences entirely uncontrolled by the nema. As far as the writer knows, different tropisms have already been claimed to be factors playing an important rôle in the location of their hosts, or special organs of hosts, by certain parasites. For example, special stress is laid on the thermotropism of the hookworm larvae. However, it seems to the writer that temperature cannot be the only directing agent. Plant-parasitic nemas also have their optimal temperature and move toward it and show therefore a thermotropism similar to that of the hookworm larvae, and yet it would be a mistake to say that thermotropism is the leading agent in host selection in this case.

It is the so-called histotropism (8, 20) of parasitic nemas which is apparently a result of a mechanism similar to that here described as the host selection mechanism of plant-parasitic nemas. This histotropism, resulting in the finding of their special host organs or host tissues by the parasitic nemas, is undoubtedly a chemotropism and the managing sense organs are assumed to be the amphids. A very definite host-tropism for certain Mermithidae will be described in a paper now in preparation.

It seems also that for certain nemas parasitic in animals, host specializations occur similar to those here described for the plant parasites. Although the matter has not yet been studied along the lines followed in the present paper, there has been much argument and discussion with regard to the identity or systematic difference of some *Ascaris, Belascaris,* and *Trichuris* living in man and domestic animals. Some investigators regard the specimens from different hosts as different species, and others do not. The situation here is not so explicit as in the plant-parasitic forms, and will still need much enlightenment. Some statements about the possibility of the transmission of a parasite from one host to another are rather contradictory, and the situation, as presented today, recalls clearly what has been said about the behavior in this respect of plant-parasitic forms (4).

THE FOOD SELECTION OF FREE-LIVING NEMAS

Comparatively few species of free-living nemas have yet been reared and are more or less known in their feeding habits. Most of these known forms are saprophytic or saprozoic feeders; a good part of them live on bacteria and their products. It is usually thought that these saprophytic and saprozoic forms can easily be reared on almost any kind of decaying organic material. Yet, anybody who tries to rear these nemas may gather experiences of a nature similar to those described in connection with the behavior of plant-parasitic nemas in choosing their hosts. Sometimes the initial culture of one of these nema species is rather difficult; they seem not to be in their optimal conditions; many may die, and only a few finally adapt themselves to the new conditions. After this the culture will go on beautifully; but if again a new kind of food is used, very often new difficulties will develop, which, however, will only last until the nemas have again adapted themselves to this new food.

We gathered some evidence of this kind in connection with *Cephalobus subelongatus* Cobb, a polyphagous species, and yet if we changed the food, after having given a certain kind for a long period, there was always a stop in the development of the cultures. Furthermore, we made similar observations with cultures of many species of *Rhabditis*, *Diplogaster*, and other *Cephalobi*, etc. We could also quote statements of the same nature from literature, as, for example, Potts (41) and Gilbers E. Johnson's (27) study of the nemas of the common earthworm. Here too the same food for a long period, e.g., for several generations, seems somewhat to specialize the nemas living on it in a way similar to that recorded above for plant-parasitic nemas.

HOST SELECTION AND HOST SPECIALIZATION IN OTHER ANIMAL GROUPS COMPARED WITH THAT OF PLANT-PARASITIC NEMAS

It is not my intention to give here an extensive review and discussion of all the known facts; my aim is only to recall certain outstanding examples which are rather parallel to the situation here treated. Unfortunately the host selection mechanism of most of the lower invertebrate parasites which locate their hosts in an active way has not yet been studied. In higher invertebrates, such as insects, etc., the rôle of organs of scent (smell or taste) is well known and is often wonderfully developed. There are examples of host selection which can be fully paralleled to that of the plant-parasitic nemas (15, 57). We refer in this connection to an interesting paper of Craighead (14) which describes the behavior of a series of Cerambycid beetles toward different host plants, based on extensive experiments. The host-selection principle, as defined by Hopkins (26, p. 353), that an "insect species which breeds in two or more hosts will prefer to continue to breed in one to which it has become adapted" can certainly also be applied to the plant-parasitic nemas, as previously shown.

The host specialization of certain Protozoa, Helminths, and Arthropods parasitic in man has recently been discussed by Chandler (11) to whom we may also refer for more literature. Chandler shows that conditions similar to those of the plant parasites here described are also found in mites and lice, in tapeworms, trypanosoms, and trichomonads, in Rickettsia-like organisms, in spirochaetes, and bacteria. There is everywhere this one problem of the systematic relationship of forms living in different hosts, differing from each other very slightly, or not at all, morphologically, but varying in infectivity for different hosts. But in all these examples from other groups the situation is not yet so clear as in the plant-parasitic nemas. In the latter we already know the identity of all these forms specialized on different hosts, whereas this identity is still in discussion in connection with the above-mentioned cases of insects, etc. The actual situation as it exists is not so little known here as there, and the plant parasites offer a much better field for investigations of the host specialization problem in general than any animal group. The problem is of highest importance, not only for parasitology in all its branches, but also for all parts of biology, applied as well as theoretical; this may be discussed in the following section.

CAUSES AND THEORETICAL SIGNIFICANCE OF HOST SPECIALIZATION

Undoubtedly the factors which are at work in bringing about all the changes (physiological and morphological) that a parasite shows in its adaptation to a new host, and in its specialization to different hosts, are of similar nature in all animal groups previously mentioned. Each new or different host represents a change in environment. All investigators agree that at least physiological changes take place, and it seems evident to me that these go hand in hand with morphological changes, although they are often hard to perceive. There are indications of morphological differences in some "strains" of our plant parasitic nemas, but they have never been studied with enough exactitude. Do these changes take place before the parasite reaches the new host, or are they a result of the new environment? Chandler in his interesting paper (11, p. 334) discusses this matter and lays much stress upon a possible segregation and survival of favored genetic races but does not see in this process the only possible way; somatic acclimatization, induction, and preinduction in his opinion, although of less importance, are still processes which nature may use here.

Chandler goes even further: he sees in the formation of many geographical and ecological races of species the same factors at work as in the process of host specialization. Very little is known about the true nature of the process, but what is known certainly puts this phenomenon close to that of host specialization.

The same author in this connection also discusses the so-called drug resistance or what Ehrlich (17) termed "Giftfestigkeit" of the lower organism. Here too, it seems to me are similar factors at work. If a certain population of these organisms can be accustomed step by step to otherwise deadly toxic substances, this phenomenon at least greatly resembles, or is perhaps identical with, the adaptation of some parasite to a new host and the gradual specialization on a certain host as shown in our nemas and as expressed in Hopkins's principle applied to insects.

The problem involved here is that of the ability of the living matter to respond to outside stimuli in an adaptive, regulative way. But it is not our intention to discuss here this question which is already fully dealt with in many other papers. The plant-parasitic nemas are exceptionally good subjects for the study of the whole problem. The situation, at least with regard to the metazoa, is here, as already stated, more explicit than anywhere else. The chief point is the fact that the changes begin with the host-selecting mechanism. As shown in previous sections of this paper, progressive host specialization in our plant-parasitic nemas begins with a progressive faculty of recognizing a preferred host, of answering the stimuli from this host in a more and more accurate way, and of being more and more sensitive to these very stimuli. At this time the parasite is still outside the preferred host and therefore not yet subject to its full environmental influences; this makes the phenomenon still more astonishing but points to a definite way of how the changes for specialization take place. The perceiving apparatus of the chemical sense organs is first involved and shows, it seems, the most outstanding changes.

There are several possible explanations. Have we here an exclusive nervous action? Are the sense organs and the conducting system only involved in the changes in that a certain kind of stimulus is more easily perceived by its repetition? Have we here a kind of ability to learn?

In the whole process one is led to see simply a result of selection. If we assume that in a somewhat polyphagous population a number of food strains are contained, then the gradual specialization on a certain host plant would be brought about by a more and more strict selection. But this explanation does not seem to me entirely satisfactory. It would be necessary to assume that even in the highest specialized population specimens of still impure genetical constitution are present from which strains of any kind could be segregated, and cases like those observed by Tischler (56) could hardly be understood. Here, if left to choose the host, the entire population goes to *Circaea lutetiana,* and no specimens to *C. intermedia.* But if there is a lack of the former, *C. intermedia* is well attacked. If selection were the only and the chief agent, this process would not go on in the manner observed; a number of specimens would certainly go to *C. intermedia* in Tischler's case where there was a choice, and it seems to me that the very same number of nemas, and no more, ought to be found on *C. intermedia* in the second alternative. Any such experiment of free choice of food and forced food should result in the same Mendelian segregation. However, since this is not the case, it seems to me to disprove the conception that selection is the chief factor.

But still another line of thought for explanation and understanding of the phenomenon might be considered. The nervous end apparatus, by the generation-long action of the same quality of a stimulus, seems to lose the ability to respond to any other quality; therefore it could be thought that the assumed ferments in the amphidial fluid get more and more adapted to the specific stimulus of the one host plant as the digestive ferments answer the nature of the food taken in. This would mean that the glandular cell, supposed to be always connected with the nervous elements, would, by generations of life on the same point, lose the capability of changing the quality of the ferment. Or does the nature of the food, after the first step in adaptation to a new host is once made, influence the supposed amphidial ferments in a parallel direction?

The whole situation, as described above, reminds one much of the behavior dogs show toward game animals. Some are specialized on rabbits, others on foxes or some other specific game, and still others take up all kinds of game.

THE HOST SELECTION AND HOST SPECIALIZATION PROBLEM IN ITS APPLICATION IN THE STUDY OF NEMIC PLANT PESTS

The facts and viewpoints described and discussed in the foregoing sections are doubtless of much practical significance, at least in the study of nemic plant pests. Several well-separated problems can now be seen; to distinguish between which is undoubtedly of value. There is first the problem of the attraction, non-attraction, and repellancy of nemas by plants. As a second problem comes that of the resistance of hosts toward the attacks of nemas. Here the nemas are attracted by the host, but mechanical, chemical, or other obstacles prevent them from entering. A third problem is that of host immunity. Here the nemas are attracted by the host and are able to enter it and live in the host, but apparently do no perceptible harm to it.

The Problem of Host Attraction, Host Indifference, and Host Repellancy

At various places we have already called attention to the value of the knowledge of the host selection problem in control of nemic pests in plants. The problem divides itself into the three subproblems named in the title of this chapter. Of these, host attraction and host indifference are closely related to each other. It is needless to repeat what has already been said about the importance of a thorough knowledge of host attraction and host indifference for any application of Kühn's trap-plant method or the "Reizpflanzenmethode" of Baunacke or crop rotation. Host attraction and host indifference are phenomenona of very variable nature. Although future investigators may have to solve numerous question, these following points are regarded as somewhat ascertained.

1. Host attraction and indifference are not only the result of the specific constitution of a plant, but also of the host history of the immediate ancestors of the nema.

2. The choice of a catch- or trap-plant, that of a "Reizpflanze," and that of a crop in crop-rotation control methods has to be based upon this host history of the nema population to be controlled.

3. Perennial plants, even though they are not yet known as hosts, should rather be excluded from crop rotation in nema-infested fields, as any nema population having the same plant at its disposal for generations may adapt itself to it.

4. In crop rotation on nema-infested fields, not only the crop history, but also the nature of the weeds present each year has to be known and considered in the choice of a suitable crop.

5. In irrigated land, hitherto it was thought that the nemas were spread by the carrying capacity of the soil water. After the experiment of Baunacke mentioned on p. 503 and after we learn that the soil water is transmitting the attracting chemical stimuli from the plants to the nemas, this problem gains a more complex aspect. Water flowing from uninfested fields to infested fields must direct the nemas of the latter to the former if the same plants, or even more preferred ones, grow there. Naturally this is true only of the water flowing in the soil itself, and not of that of the watering channels.

If an isolation and determination of the chemical substances which act as attracting stimuli upon the nemas could be made, the way to the use of baits would be more easily approached, and perhaps more economical and efficacious methods than the catch-plant method of Kühn could be found. A recent publication by Rensch (46), as already mentioned, opens wide possibilities along these lines.

With regard to the third sub-problem here touched upon, that of host repellancy, there are very few facts known. Chicory and onions are said by some authors (25, p. 8; 29, p. 334; 35, p. 1007; 36, p. 89; 64, p. 8) to act in a way which at least partly resembles repellant action.

If plants could really be found which are repellant toward the nemas above mentioned, it would not seem impossible to find out the cause, and perhaps the chemical, which acts as such.

Hitherto investigators have been most often endeavoring to find chemicals to kill the nemas in the soil. Although hundreds of different preparations were tried, no very satisfactory result was obtained. A search for repellants has not yet been made. Perhaps such fluids could also be found for plant-parasitic nemas, as the anthelminthics used for removing *Ascaris* (Santonin, Chenopodium) or hookworm (carbon tetrachlorid) are perhaps repellants, and not killing fluids. The ideal arrangement would be to have a repellant which would be highly parasitotrop (17) and would act at the same time as a fertilizer on the plant so that its repeated application would result in a doubly beneficial influence on the plant.

The Problem of Host Resistance

In the past, investigators spoke very often of host resistance in cases where non-attraction would have been the right term. A plant can only be termed resistant if it actually resists the attacks of the nemas. But if the latter do not attack it because they do not perceive its presence, such a condition can hardly be called resistance. In the study of plant-parasitic nemas, the resistance of a plant means that it opposes the entrance of the nemas by some mechanical or perhaps chemical means. The use of resistance in this restricted sense is not only more appropriate in the existing situation but also helps to separate the wide problem of mutual relationship of plants and nemas into smaller parts with more definite questions and more sharply outlined points of attack. Heretofore, if a plant was not found to be infested, it was termed resistant, regardless of the causes, which often were not at all within the plant.

A plant may resist the attacks of nemas either by some mechanical or chemical means. Unfortunately very little is known with regard to this. There are a few definite cases of apparently real resistance mentioned in literature (62, 32, 39, 63).

Only for cowpeas, as far as the writer knows, was an attempt made to find out the cause of resistance. Through the courtesy of Dr. Arzberger,[2] the writer had the opportunity to read an unpublished paper on some investigations regarding the causes of the nema resistance of several cowpeas. The results were that of five studied varieties, the more resistant, Iron and Brabham, had the roots best guarded by protective tissue; their cork layer was better developed and had fewer broken areas; the cork cells had more suberized walls, and the mechanical tissue in the cortex was, on the whole, more uniformly distributed. Furthermore, the cells containing starch were more remote from the periphery of the root, and starch was not so abundant as in the cortex of the non-resistant varieties. It will be one of the first tasks in coming investigations to define more clearly the causes of the restistance of plants as outlined above. It might then be difficult sometimes to distinguish true resistance from immunity. A number of similar cases are known, however, for insects (see 40, 48, 58, and others).

The Problem of Host Immunity

If we compare different nema-infested plant species, we notice that the degree in which they suffer from attacks is quite variable. One plant species will suffer much from a light attack and even be killed, whereas another species will show, in even a heavy grade of infestation, no signs of real suffering. In this connection one of the many examples in literature is cited. *Papaya gracilis* seems to suffer more and to react more differently from the attacks of *Heterodera radicicola* than numerous other plants.

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[2] Arzberger, E. G. A comparative morphological study of cowpea roots resistant and non-resistant to nematode infestation. [Unpublished.]

According to Beille (5), the swellings here reach only the size of a hazel nut and then decay, whereas other plants, for example, *Dioscorea illustrata*, according to Queva (42), seems to be absolutely indifferent, in that infested specimens show no difference from the uninfested ones, and in some cases *H. radicicola* even seems to be useful to the host. This is stated by Vuillemin and Legrain (61) for a number of crop plants in the dry soils of the oases in the Sahara. These plants lignify the walls of the giant cells due to the attack of the parasite and use the so-formed cavities as water reservoirs. This makes them able to accumulate water for the hot part of the day and thus to withstand the dryness, while specimens not harboring *H. radicicola* perish. Here the parasitism is changed into a useful symbiosis. Here, again, is an entirely separate problem, that of host immunity. In the study of the nemic pests the phenomena of non-attraction, host indifference, host repellancy, and host resistance were all termed immunity, despite the fact that they are very different phenomena. The immunity problem involves the whole behavior of a plant actually under influence of the attacking nema. To separate this phase of the mutual relationship of plant and nema is not only an aid in allowing the investigator a much more precise outline of all questions to be studied, but is an actual progress in the knowledge of the matter itself.

In the study of this immunity problem, it is again the morphology of the nema which gives the basic facts and conceptions. Many plant infesting nemas possess relatively huge so-called salivary glands. On the other hand, botanists and other investigators with very little knowledge of a nemic organism claim that the plant attacked is under the influence of some secreta of the nema.

A third point is that the spear present in these parasitic nemas is said by some investigators (6) not to be a means for actual attack and puncturing of the plant cells, as first was thought. Observations show that this spear is only a trifle protruded, its action being a quickly repeated running back and forth so that the point hardly comes out of the mouth opening. Furthermore, the spear is often so narrow and fine that the conception of its being the direct means of the destruction and swallowing of plant cells and their contents must be dismissed. A study of attacked plant tissue also shows that no cells are emptied or destroyed in a mechanical way (38, pp. 151–173).

The action of the nema on the plant is apparently as follows: The nema ejects into the tissue of the plant by means of a short but rapid back-and-forth movement of its spear a liquid substance, presumably a secretion of the strongly developed salivary glands. Under the influence of this secretion the plant reacts in various ways, forming the giant cells (nectarial cells) (38) and tumor-like outgrowths, fluid exudates, etc. Perhaps also the excreta of the parasites living entirely inside the plant may contribute stimuli to reactions, or at least influence the plant cells in their behavior. The so-called giant cells are formed only just around the mouth opening of the parasitic *Heterodera*, showing with evidence that they must be formed under the influence of an agent coming from the mouth opening. The careful examinations of the plant tissues showed no mechanical injuries to these cells, so apparently the spear itself is not the cause. What can it be therefore but the secretions from the huge salivary glands of which the outlet leads directly behind the spear into the oesophageal tube?

It seems to me, therefore, that there is much evidence that the harm these plant-parasitic nemas do to their host in the first degree is not mechanical, but toxic. The plant species, however, seem to react quite differently toward these toxins; and here is the point where the immunity problem begins. Have certain plants substances to bind, to neutralize these toxins? Are there antibodies present? We do not know, but future investigators will have to keep this problem in mind. It is not only of theoretical, but also of practical, interest. Might it not be that such knowledge could lead us to therapeutical treatments of nema-infested plants? If an annual crop plant is suffering under nemic infestations, there is at least this way to kill the plant and with it the harbored pest. But applied nematology has as its goal also the cure of perennial plants, such as fig trees, citrus, peaches, vines, etc., to which such radical killing methods can not easily be applied. To avoid large financial losses, it seems that only theropeutical methods might be possible, although plant therapeutics are today still a field hardly touched (16, 46, 7), but there is no reason for the conception that they are impossible. In the literature chiefly about *Heterodera schachtii* we find mentioned again and again that potassium has a very beneficial effect on plants (sugar beets) attacked by *Heterodera*. This point stands out so well that at first the reason for the bad growth of infested sugar beets was thought to be a scarcity of potassium in the soil. Today, the connection of potassium with a better growth of Heterodera-infested sugar beets has not been cleared. This connection is apparently not only a purely nutritive effect which the potassium has, but it also points toward a certain neutralization of the toxic influence of the parasites. If that is so, might it be possible in the future to step forward and find substances being fully parasitotrop (nematrop) and little, or not at all organotrop?

SUMMARY

1. The question is raised how the soil nemas, especially the plant-parasitic forms, locate their food, their host plants. A number of examples and observations are given which show the ability of the nemas,
    a. to distinguish their hosts at considerable distances and to locate them in an active way;

   b. to distinguish between the different hosts and to choose the one they prefer.
2. But it is shown that the behavior of different populations of one plant-parasitic nema species toward the same host plant is variable, often showing gradual differences from heavy to light attacks, sometimes even to an imperceptible infestation.
3. It is shown that these differences in infestation of the same host plant by various populations of the same nema species are a result of conditions which lie within the nema and not within the plant, and that the situation is as follows:
   a. A plant-parasitic nema apparently prefers always the host species or even the host variety on which its parents lived; the host principle, as established by Hopkins for insects, is also applicable to our plant-parasitic nemas.
   b. This preference for a certain host grows with the number of generations a nema population lives on it, the latter getting more and more specialized on this very host. This specialization may reach such a high degree that finally even new hosts of the closest taxnomical, physiological, and chemical relationship to the old host are attacked no more or very lightly.
   c. In the judgment of the behavior of a nema population toward its host, the former host history is always of primary importance and in addition to this also, the possibility of choice. Forced by starvation, a nema population will attack a host which it will completely ignore if a more preferred one is present.
4. The mechanism of this host selection is outlined, based upon the observations and experiments of Buerkel, Baunacke, Rensch, and others, and a close study of the morphology of the sense organs of the involved plant-parasitic forms. It is shown that this mechanism works apparently as follows. The growing plants produce some root secretions which are carried by the soil water and act as stimuli upon the nemas The latter apparently perceive the stimuli with a sense organ called amphid or lateral organ which is thought to be a chemical sense organ. Presumably with the aid of these amphids the nema moves toward points of higher concentration of the stimulating fluid and so finally locates its host. The structure of the amphids of a few plant-parasitic nema species is for the first time described and their importance in general for the nemas discussed.
5. The dominating significance of the host selection mechanism, as outlined, is shown:
   a. in its power to explain fully the otherwise puzzling behavior of the nemas towards their hosts, a behavior which in the past resulted in so many seemingly contradictory observations.

b. in its practical importance for various control methods, such as Kühn's trap- or catch-plant method, Baunacke's "Reizpflanzen" method, Rensch's recently developed activation method of the cysts of the sugar beet nema, the crop-rotation method, and furthermore for all studies on host immunity, host resistance, host attraction, host indifference, and host repellancy.

c. in its theoretical significance for the conception of species, varieties, races, and strains of the nemas involved.

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PRESENCE OF THE EUROPEAN BROWN-ROT FUNGUS IN AMERICA

Walter N. Ezekiel

With Three Figures in the Text

The presence on the Pacific Coast of a Monilia disease differing from the common brown-rot was first recorded in 1915 by Jackson (6), who described a pear disease caused by an unidentified *Monilia*. This was studied by Posey (9, 10) who found it different from "*S. cinerea*" elsewhere in the country, and from *S. fructigena* from England. Barss has described the typical blossom and spur blight injury caused on stone fruits and some varieties of pears; and, chiefly on the basis of Posey's work, proposed for the fungus the name *Monilia oregonensis* Barss and Posey (2, 3). More recently Rudolph described the Monilia blossom blight of apricots, destructive in California, with extensive investigations and directions for its control (13).

In a more detailed paper (4) the writer has given the results on which was based the conclusion (7) that the widespread and destructive brown-rot *Sclerotinia* common in this country, to which the name *S. americana* (Wormald) Norton and Ezekiel[1] has been applied, is specifically distinct from *S. cinerea* (Bon.) Schröter of Europe. In the course of this work some cultures from California and Oregon have been of special interest. The California cultures studied were from rotten apricot (S 45), peach (S 46 and S 47) and cherry (S 49) sent by Mr. B. A. Rudolph during the summer of 1923. Prof. H. P. Barss kindly furnished a culture of the Oregon spur-blight *Monilia* (S 56). These were compared with a large population of other single-spore cultures, also collected in this country and now all assigned to *S. americana*, and with *S. cinerea* and *S. fructigena* cultures from England and Holland. Of the California and Oregon strains mentioned above, all except that on apricot were found to be the true *S. cinerea*.

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[1] As Pollock (8), Roberts and Dunegan (12) and the writer (4, p. 89) have previously pointed out, the name *S. fructicola* (Winter) Rehm was the first to be applied to what were probably apothecia of *S. americana*. If means of distinguishing *S. americana* from *S. cinerea* by the apothecial characteristics should be worked out, we might then be able to identify the dried specimens of Winter (15) more definitely with *S. americana*, which would then properly be called *S. fructicola*. For the present, it seems preferable to use Wormald's name *americana*, which was published with the first description (16) that differentiated the American species from the earlier known European forms.

CHARACTERIZATION OF S. AMERICANA AND S. CINEREA[2]

It would be needless to mention here all the experiments previously described (4) in which consistent differences have been demonstrated between the range of varieties of *S. americana,* on the one hand, and *S. cinerea*[3] strains, from all the sources mentioned, on the other; but it may be of interest to outline the methods that have since been found most convenient for their differentiation. (The use of type cultures for comparison is an advantage in connection with the tests which follow but is not indispensable.)

Cultural Characteristics in Potato Dextrose Agar Slants at 15–25° C. Strains of *S. cinerea* grow slowly and the surface of the colony is characteristically smooth and buffy brown to Saccardo's umber (11) in color, though with some strains it may be partly or entirely white. Conidia are absent or produced only sparsely and never in definite pustules, visible macroscopically, in any of the strains yet studied by the writer. On the other hand, almost all strains of *S. americana* produce abundant conidia in definite (Tilleul buff) pustules. Absence of such pustules is not necessarily an indication that the culture is of *S. cinerea,* since the more uncommon varieties of *S. americana,* var. V and VI (4), produce few or no conidia under these conditions; however with the common varieties of *S. americana* abundant development of conidia affords ready recognition by this method.

Petri Dish Cultures on Potato Dextrose Agar. *S. americana* grows rapidly, filling the whole plate with a colony homogeneous except for numerous concentric circles of conidia, or in some varieties, of aerial hyphae. With *S. cinerea* growth extends much less rapidly, and is characterized by zonation and lobing as shown in fig. 1. Conidia if present are not borne in definite pustules as is invariably the case with *S. americana,* but scattered around so that the surface of the colony may be smooth and of the characteristic dull-colored, somewhat velvety, appearance.

Growth in Drop Cultures. Growth in standardized hanging drop cultures furnishes a number of criteria for identification. The results discussed below have been obtained under these conditions: potato dextrose decoction (potatoes 200 gm., dextrose 10 gm., per liter) was used as the nutrient; drops were made with a 5 mm. loop, seeded with 30–50 conidia, sealed on shallow depression slides and incubated at 25° C.

In 15 to 18 (perhaps best in 16) hours after inoculation, observations of typical *S. americana* sporelings show only the original germ tube, generally with no side branches, or if branched the branches are small and distinctly subordinate. Though there may be local irregularities in this germ tube it is straight in its general direction of growth. With *S. cinerea* on the contrary, total linear growth is less (100 to 300 μ, while *S. americana* germ tubes are 500 to 1000 μ long), there may be three or four branches and the branches are characteristically of equal dimensions so that it is difficult or impossible, even in so young a colony, to pick out the original germ tube (fig. 2). Branching is almost invariably dichotomous, neither branch following the former direction of growth of the branching hypha so that a network of twisted, contorted, mycelium results.

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[2] It will be noted that the differences mentioned between these fungi include substantially all of those first observed by Wormald, except that of differential production of oxidizing enzymes, as they proved of diagnostic value, with the modifications noted, throughout the large population studied by the writer.

[3] In the present paper, discussion of *S. cinerea* applies primarily to the forma *pruni* of Wormald.

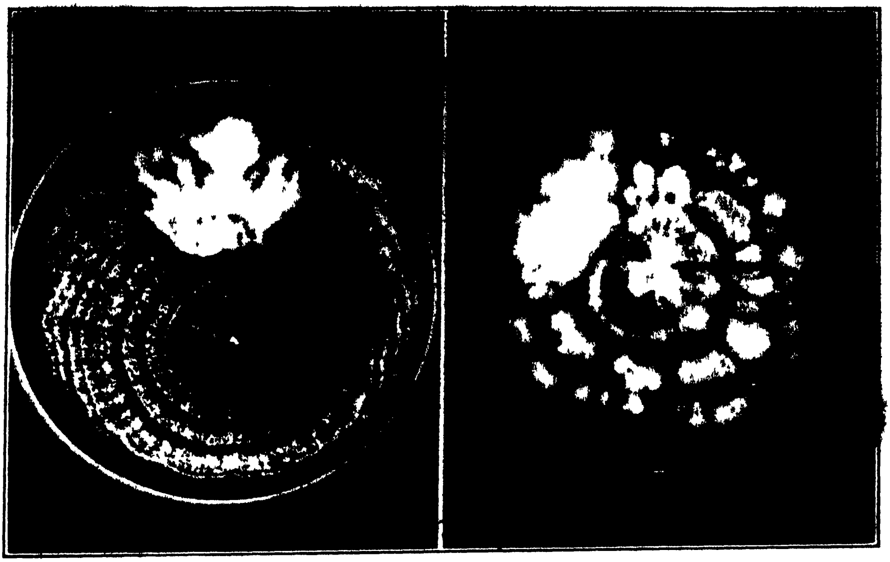

FIG. 1. Petri dish cultures on potato dextrose agar. Left, *S. cinerea* forma *pruni* (S 44) from England, upper colony and *S. americana* var. I (S 22) lower colony; inoculated at the same time at the same distance from the center. Grown 11 days, 13.5° C. Right, S 47, from California, one month at room temperature of about 15° C. [Direct prints (negatives) of the same cultures shown in regular photographs in Md. Bul. 271 (4), figs. 8 and 9.]

Observations after three days yield equally definite differentiation. Fig. 3 shows the general appearance of the mycelium of *S. cinerea* and *americana*, which is itself distinctive. The *S. americana* hyphae are long and straight, and branching is simple—in glancing over the slide it is possible to pick out at once the older hyphae from which the branches arise. With *S. cinerea* the mycelium is of a much denser and more homogeneous nature. Each hypha arises from dichotomous branching. All twist and bend so frequently that it is unusual for a hypha to be found that is straight for even the distance of a few fields of the microscope. This is markedly different from the *S. americana* hyphae, which can be traced straight, all the way across the drop.

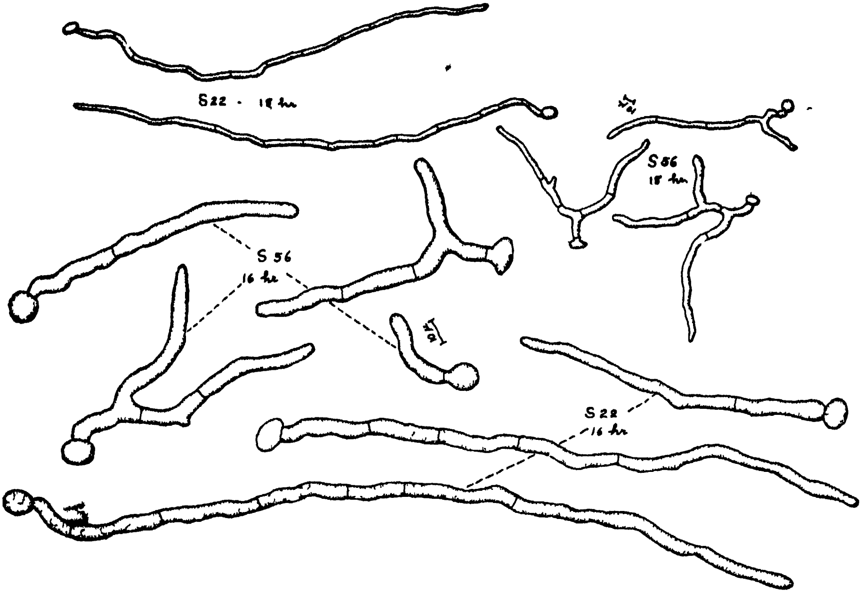

FIG. 2. Conidia of *S. americana* from Maryland (S 22) and *cinerea* from Oregon (S 56) germinating in drop cultures of potato dextrose decoction at 25° C. Note the characteristic geniculate branching in S 56. [Outlined with camera lucida.]

An additional distinction is the average length of the cells of the older hyphae. Growing under the standardized conditions mentioned, 19 sets of measurements, each including 25 cells of the larger hyphae of various strains of *S. americana* gave a mean value of 66 μ. A similar series of 10 sets of measurements of *S. cinerea* strains averaged only 36 μ.

In some recent series of drop cultures, with strains of previously well-established identity, atypical growth has at times been secured. Though this is unusual the fact that it has occurred indicates that the conditions specified for observation are not, as at first believed, sufficiently definite to allow us to consider the growth in single series of drop cultures, alone, the basis for differentiation. Until the method can be still more precisely worked out, perhaps with inorganic media, it would seem preferable to use it in connection with the cultural characteristics. In practice, microscopic identification in drop cultures has always agreed with the less laborious, macroscopic, cultural identification; and the former can doubtless be omitted except in doubtful cases.

IDENTITY OF THE PACIFIC SLOPE BLOSSOM BLIGHT MONILIA

The cultures from California and Oregon, mentioned before, fitted in with the *S. cinerea* cultures from Europe exactly, by these tests as well as in various inoculation experiments, spore measurements, etc. In drop cultures the results were identical. Their cultural characteristics on solid and liquid media, in tubes, agreed throughout. While constant individual differences could be seen in Petri dish cultures, the series as a whole agreed exactly with the series of strains of *S. cinerea* from Europe. Individual variation noted was hardly of a greater degree than that previously described between cultural varieties of *Sclerotinia americana.* Accordingly, it seems proper to designate these Pacific Coast cultures as *Sclerotinia cinerea*

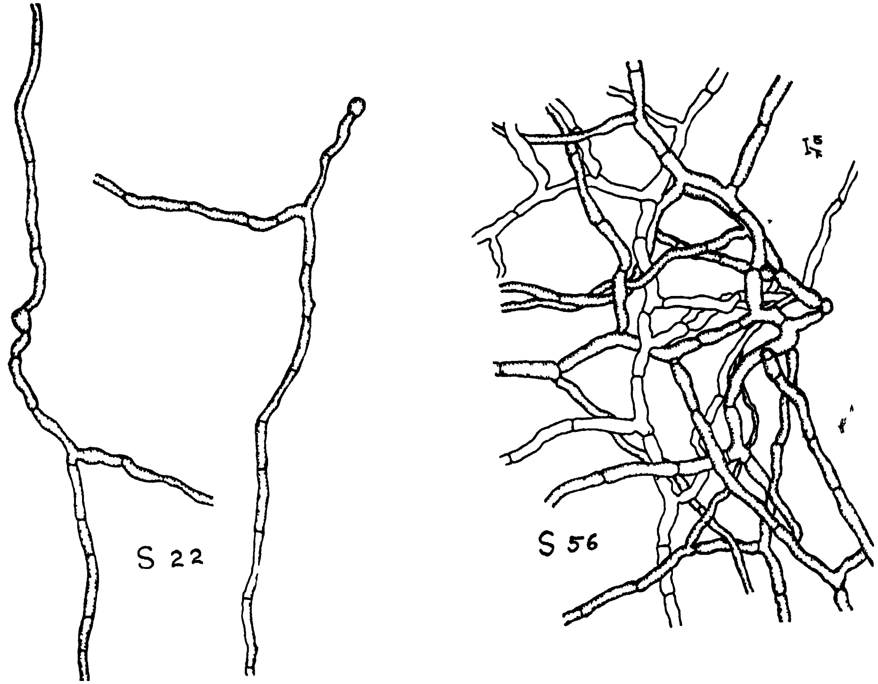

FIG. 3. General mycelial characteristics in two day old drop cultures of *S. americana* (S 22) and *S. cinerea* (S 56). Growth is more extensive with *S. americana* but very loose, while with *S. cinerea* a great amount of branching occurs though the entire colony is small. [Outlined with camera lucida.]

This conclusion is of especial interest in connection with the Oregon fungus. This culture was supplied by Prof. Barss, who has recently discussed the life history of this organism and considered it distinct from all described species. Though as shown also by the studies above the organism is quite different from *S. americana*, it resembles rather clearly the earlier-known *S. cinerea* of Europe; and the life history which Barss cites to differentiate his *M. oregonensis* from the ordinary brown-rot fungus is a further resemblance to *S. cinerea;*

(1) The apothecial stage of *S. cinerea* has been authentically found only by Wormald (17), unless the *S. laxa* of Aderhold and Ruhland (1) or the *Sclerotinia* on cherries of Westerdijk (14) can be assigned to that species. Similarly, Barss reports that the Oregon fungus failed to produce apothecia under experimental conditions resulting in abundant development of *S. americana* apothecia.

(2) While the Oregon *Monilia* produces conidia much like those of *S. americana*, they develop much more sparingly in culture, and on twigs and flower parts are produced mainly, not during the summer following infection, but late in winter or early the following spring. Here again, *S. cinerea* is described by Wormald (18) as producing pustules of conidia on the bark of shoots or cankers on plum trees only from about December to February.

A similar relation was developed experimentally by the writer in inoculations on fruits in the laboratory (4, p. 119). Generally strains of *S. cinerea* produced no conidia while those of *S. americana* produced amounts varying with the different strains; but in an extensive series on Shockley apples no conidia developed away from the point of inoculation with any *S. americana* strains, while pustules were produced on most of the *S. cinerea* inoculations, which included two of the California strains (S 47 and S 49).

(3) The Oregon fungus is found more frequently on blossoms and twigs and causes "a negligible amount of fruit rot." This is not unlike *S. cinerea* which has attracted much more attention in Europe as a parasite of the woody part of the tree than *S. americana* has in this country. It is perhaps hardly remarkable that little infection on fruit occurs when one considers that the organism generally produces conidia before blooming and not after fruit are developed. Moreover, the slow rate of growth of *S. cinerea* in infected fruits, as compared with that of *S. americana*, coupled with the absence or scarcity of conidia on them during the summer, might tend to lessen the chance of such specimens being collected and studied. (This last point has come up in connection with our survey of the Maryland brown-rot diseases.)

(4) Barss considers artificial cultures of the Oregon fungus different from those of any described species. This is of course true in the sense that each isolation of the brown-rot fungi results in a strain of unique cultural characteristics, and distinguishable by sufficient study from any other strain. However in the cultures studied by the writer, S 56 from Oregon resembled much more closely a culture received from Wormald, S 44, than

the latter did S 47 from California, a strain agreeing exactly with Wormald's description of *S. cinerea* cultures.

PATHOLOGICAL SIGNIFICANCE OF THE PRESENCE OF S. CINEREA IN AMERICA

Diseases due to what is probably *S. cinerea* have been studied in Oregon (2) and California (13) where the predominant *S. americana* is also present. Barss, who distinguishes between the two organisms, attributes greater injury in the form of blossom and twig blight to the *S. cinerea*, but finds it of less economic importance because it causes little injury in the form of fruit rot as compared to *S. americana.* On the other hand, *S. cinerea* is found to attack not only stone fruits but also some varieties of pears.

SUMMARY

1. Isolations from fruits from California, and the spur blight *Monilia* from Oregon, were identified as the true European brown-rot fungus, *Sclerotinia cinerea* (Bon.) Schröter.

2. Methods are outlined by which *S. cinerea* can be distinguished, by cultural characteristics as well as microscopically by numerous mycelial characters in drop cultures, from *S. americana,* the species occurring predominantly in this country.

3. *Monilia oregonensis* Barss and Posey agrees morphologically, culturally and in its life history with *S. cinerea,* and is doubtless to be considered synonymous.

4. *S. cinerea* causes blossom and twig blight injury, with little fruit rot, to a wide range of hosts. It has not been considered so destructive as *S. americana* in reports from regions where both occur.

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STUDIES ON THE MOSAIC DISEASE OF NICOTIANA GLUTINOSA

M. N. WALKER[1]

WITH PLATE XXIV

INTRODUCTION

A great amount of cross-inoculation work during the past few years has pointed to a common casual agency for many of the mosaic diseases, and the apparent existence of two distinct mosaic diseases within the single genus *Nicotiana* led the writer to the further investigation which this paper reports.

LITERATURE

The literature of direct bearing on the question consists of two papers by Allard. The first paper appeared in 1916 (2), giving data which indicated that a mosaic disease on *N. viscosum* was distinct from that on tobacco, *N. tabacum* L. A later paper which appeared in 1917 (4) merely mentioned the existence of a mosaic disease on *N glutinosa L.*, which was distinct from that on tobacco. The similarity in literal meanings of the specific names of these two species of *Nicotiana*, said to bear mosaic diseases distinct from tobacco mosaic, led the writer to inquire of Dr. Allard whether the species were identical. Dr. Allard explained that through error the term *Nicotiana viscosum* had been used in place of *Nicotiana glutinosa*, and that the correct name had been substituted in the second paper. Dr. Allard also stated that he had at no time worked on *N. viscosum*, and kindly enclosed a description of the two distinct species which follows. In this description *N. viscosa* is substituted for *N. viscosum* as a preferred spelling.

"*Nicotiana glutinosa* L. Leaves long petioled, cordate, entire, villous-pubescent. Flowering racemes secund or one-sided. Calyx more or less bilabiate. Lowermost usually bifid, the uppermost trifid. Corolla sub-ringent, twice as big as the calyx. Stamens un-[illegible], tomentose at the base.

"*Nicotiana viscosa.* Stems viscid-villous, erect, angulate branched. Branches axillary, short, with flowers at the apex. Leaves serrate, entire, repand; lowermost and middle cauline somewhat wedge-shaped, obtuse, narrowed toward the base then becoming dilated and half clasping. Racemes sub-corymbose terminating the stem and branched. Calyx lobes unequal, short, obtuse. Corolla funnel-form, tube cylindrical slightly enlarged, about twice as long as the densely hairy calyx. Leaves average only two or three inches long by two-thirds inch in width."

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[1] The writer is indebted to Dr. S. P. Doolittle, of the Office of Cotton, Truck, and Forage Crop Disease Investigations, for suggestions and advice during the progress of this work.

The plants used in the writer's experiments agree perfectly with the description of *N. glutinosa* (Pl. XXIV, A).

DESCRIPTION OF THE SYMPTOMS OF THE MOSAIC DISEASE OF NICOTIANA GLUTINOSA

Possibly on account of the less succulent nature of *N. glutinosa,* the symptoms of the mosaic disease of this species are less striking than those appearing on the more succulent leaves of tobacco. In early stages of the disease, the tipmost leaves assume a faintly mottled appearance, sometimes so faint as to be scarcely discernible. These early symptoms may gradually become more intense until a decidedly mottled, dwarfed, and malformed plant (Pl. XXIV, B and C) may result, or they may gradually fade until the plant shows only the faintest possible traces of mottling. It has been found that when inoculations are made during favorable weather conditions, the average incubation period is about ten days.

RESULTS OF CROSS-INOCULATIONS FROM MOSAIC TOBACCO TO N. GLUTINOSA

In these experiments, all inoculations were made by inserting crushed leaf tissue from mosaic tobacco plants into stems and leaves of healthy young plants of *N. glutinosa.* On account of the stickiness of the leaves of *N. glutinosa* it was impossible to use aphids as a means of inoculation.

The *N. glutinosa* seedlings used for inoculation were all healthy and showed no signs of mosaic infection up to the time of inoculation, when they were in the four or five-leaf stage. As there were no mosaic *N. glutinosa* plants in the greenhouses during the original inoculations, the infection of the young *N. glutinosa* plants undoubtedly resulted from the inoculations. This fact was made even more certain by the fact that during three years of observation in the greenhouses no insects have ever been known to attack plants of this species.

The results of these inoculations are shown in table 1.

RESULTS OF CROSS-INOCULATIONS FROM MOSAIC PLANTS OF N. GLUTINOSA TO TOBACCO

The fact that tobacco plants are susceptible to the mosaic disease of *N. glutinosa,* as shown in table 2, further indicates the probability that the mosaic diseases of these two hosts are identical. Time did not allow the carrying out of a very extensive series of inoculations from *N. glutinosa* to other plants, but a few small experiments furnished the following results. Two tomato plants out of eight became infected when inoculated with crushed leaf tissue from mosaic *N. glutinosa* plants. Infection also was obtained in a small number of inoculations from mosaic plants of the cultivated ground-cherry to healthy young *N. glutinosa* plants.

TABLE 1.—*Results of inoculations from mosaic tobacco to Nicotiana glutinosa*

Date inoc.	Source of inoculum	No. plants inoc.	No. plants mosaic	Date of observation
5/30/23	Crushed leaf tissue from mosaic tobacco	6	6	6/11/23
5/30/23	Crushed leaf tissue from healthy tobacco (Control)	6	0	6/11/23
7/24/23	Crushed leaf tissue from mosaic tobacco	16	8	8/ 6 /23
7/24/23	Crushed leaf tissue from healthy tobacco (Control)	16	0	8/ 6 /23
7/25/23	Crushed leaf tissue from mosaic tobacco	16	8	8/ 6 /23
7/25/23	Crushed leaf tissue from healthy tobacco (Control)	16	0	8/ 6 /23
8/ 6 /23	Crushed leaf tissue from mosaic tobacco	25	11	8/28/23
8/ 6 /23	Crushed leaf tissue from healthy tobacco (Control)	20	0	8/28/23
4/29/24	Crushed leaf tissue from mosaic tobacco	16	2	5/19/24
4/29/24	Crushed leaf tissue from healthy tobacco (Control)	16	0	5/19/24

TABLE 2.—*Results of inoculations from mosaic N. glutinosa to tobacco*

Date inoc.	Source of inoculum	No. plants inoc.	No. plants mosaic	Date of observation
6/14/23	Crushed leaf tissue from mosaic *N. glutinosa*	12	2	7/ 2 /23
6/14/23	Crushed leaf tissue from healthy *N. glutinosa* (Control)	12	0	7/ 2 /23
2/28/24	Crushed leaf tissue from mosaic *N. glutinosa*	40	5	3/15/24
2/28/24	Crushed leaf tissue from healthy *N. glutinosa* (Control)	24	0	3/15/24
2/28/24	Crushed leaf tissue from mosaic *N. glutinosa*	6	2	3/15/24
5/20/24	Crushed leaf tissue from mosaic *N. glutinosa*	9	3	6/ 6 /24

DISCUSSION

In the course of this work it was found that during midwinter *N. glutinosa* plants did not show the decided symptoms which appeared during the spring months, and, in fact, several series of inoculations made during midwinter were discarded by the writer on account of the doubtful nature of the symptoms. Some of these plants when set aside, however, developed definite mosaic symptoms several weeks later. A thing of this sort has probably occurred in other plants, for some mosaic diseases have been reported to have a much longer incubation period than that later accepted, as workers by longer acquaintance with the disease were able to diagnose the fainter symptoms. This failure to produce symptoms during midwinter was probably due to a condition of the host which prevented the manifestation of decided and recognizable symptoms, as the short and often cloudy days of midwinter retard the growth of plants to a decided extent. These points have been suggested in connection with plants other than *N. glutinosa,* as for example, *N. glauca* (1, 3), *Datura stramonium* (2), tomato (4, 7), petunia (3), physalis (8), *Phytolacca decandra* (6), and various *Nicotiana* species (5).

Nicotiana glauca presents a case analogous to the one in hand. Owing to the very faint manifestation of symptoms, it was at first thought that this plant was immune to tobacco mosaic, but it was later shown that it is susceptible (3). The experiments with this species were identical with those first described in connection with *N. glutinosa.*

In view of the observations on the failure of *N. glutinosa* to show symptoms following inoculation with tobacco mosaic in midwinter, and the fact that Allard's tabulated experiments were made between December 16, 1915, and January 29, 1916, the writer is inclined to believe that the data advanced by Allard does not necessarily conflict with the present work, and to believe that the mosaic diseases of tobacco and *N. glutinosa* are probably identical.

SUMMARY

1. The Nicotiana species called by Allard *Nicotiana viscosum* is properly called *Nicotiana glutinosa.*

2. The mosaic disease on tobacco is transmissible to *N. glutinosa* and the mosaic disease on *N. glutinosa* is also transmissible to tobacco.

3. Tomatoes are susceptible to the mosaic disease on *N. glutinosa.*

4. *N. glutinosa* may be infected with the mosaic disease on *Physalis pubescens.*

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WASHINGTON, D. C.

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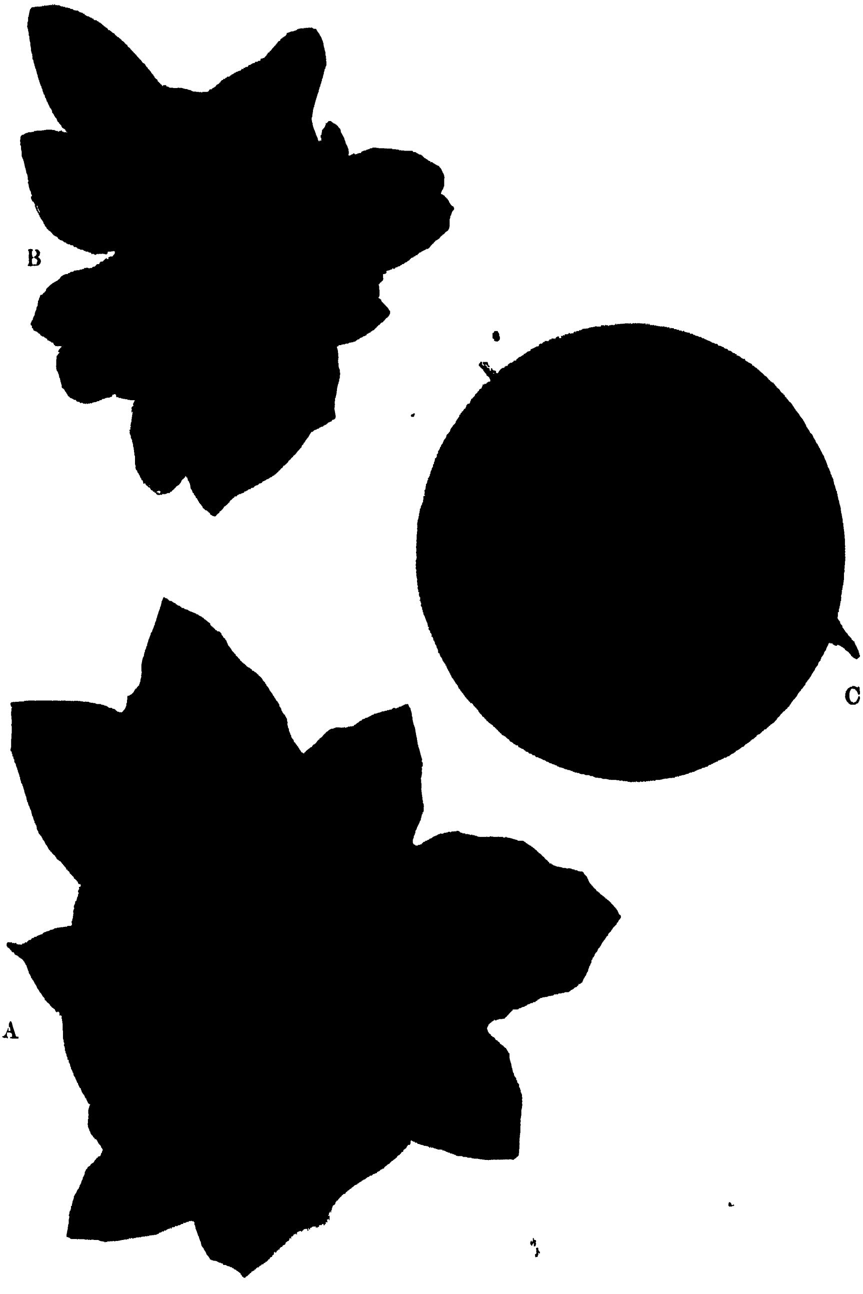
EXPLANATION OF PLATE XXIV

A. Healthy plant of *Nicotiana glutinosa*.

B. A mosaic *N. glutinosa* plant showing dwarfing and mottling. This plant also shows the fading out of symptoms. It will be noticed that the tip leaves are less mottled than some of the older leaves, a reversal of usual conditions.

C. A mosaic *N. glutinosa* plant showing dwarfing and malformation.


B
C
A

AN EFFECT OF DROUGHT IN THE FORESTS OF THE SIERRA NEVADA

E. P. MEINECKE

WITH TWO FIGURES IN THE TEXT

In the month of September, 1924, an alarming loss in seed trees left on a large timber-sale area was reported from the Stanislaus National Forest, California. Trees standing in groups scattered over the area were first observed to turn color in the beginning of September and within a very short time were unmistakably dying.

The area was visited and examined in the second half of September. The timber-sales area is located in the main on the north side of a sharp ridge sloping steeply into the Middle Fork of the Stanislaus River, at elevations between 4,000 and 6,000 feet. The forest type on the dry slopes is the usual one characterizing the granite hogbacks branching off east-west from the main backbone of the Sierra Nevada. Western yellow pine (*Pinus ponderosa*), sugar pine (*P. lambertiana*), white fir (*Abies concolor*), incense cedar (*Libocedrus decurrens*) with some Douglas fir (*Pseudotsuga taxifolia*) are the chief components of the association in which scattered California black oak (*Quercus californica*) occupies a subordinate position. Climatologically the region is characterized by cold winters with heavy rain and snow, and hot, dry summers during which all vegetation depends for its water requirements on the supply stored and moving in the soil, without replenishment by summer rains.

It is apparent at first glance that the dying goes far beyond what could be classed as normal loss. All classes, from young trees of small pole-size up to larger individuals, are involved, including in one case a sugar pine of 45 inches D. B. H. Typically the trees die in groups of from 4 to 8 and 10 trees. The direct loss in timber values is considerable and the silvicultural effect on the remaining stand is in many places decidedly detrimental. Bare places are enlarged and the objects of marking are frequently thwarted by the breaking up of the groups left.

The dying is not confined to the cut-over land but also occurs in the virgin forest. Although it is decidedly commonest on the timber-sales area in question, groups of dying and dead trees were seen two miles from the nearest cutting and a few scattered groups were observed at greater distances.

The most striking characteristics of the killing are that the trees die from the top down and from the outside in, and that they die in groups, often composed of two or more of the following species: yellow pine, sugar pine, white fir, and even black oak. The trees take on a fading dirty-green color which later tends to become reddish, and the needles look dry and wilted. The groups with their grey and discolored crowns stand out strongly against their vividly green surroundings. It is to be noted that none of the incense cedars associated with the dying trees of the other species showed any signs of distress. Several yellow pines, sugar pines, and white firs, but none of the oaks, were felled and partly peeled off for the purpose of examination.

In all the trees, with exception of the oaks, insects were plentiful at the time of cutting. Insects taken from these trees and later examined by the Office of Forest Insect Investigations were found to be species that would not be expected to cause the simultaneous dying of trees of different species and sizes in well defined groups.

The whole aspect of the trees pointed to the probability that the seat of the trouble lies in the lowest part of the bole or in the root system. Digging and exposure of the root system for examination was not feasible on account of the large size of the trees. No sign of any fungus which could have brought about the dying was found. The probabilities, however, speak rather against root fungi being at the bottom of the trouble. Both *Fomes annosus* and *Armillaria mellea* (or *Pholiota aurivella*) are known to spread from one tree to another, but it is not likely that all the trees of different species composing the group should die at the same time. It is also little probable that these fungi should cause the simultaneous death of trees differing so considerably in age and size.

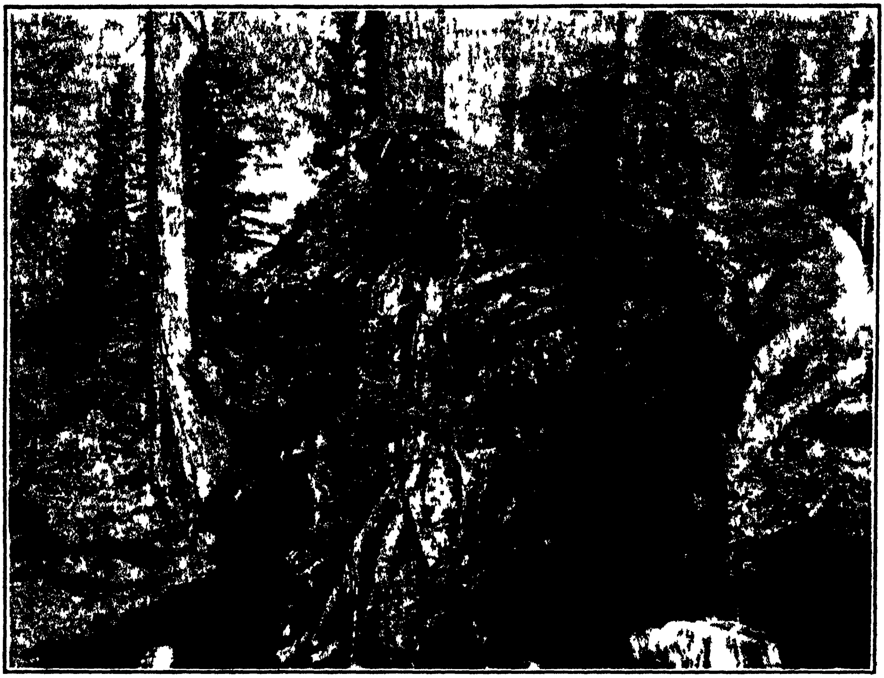
Lightning, as one of the known causes of group-dying, can well be excluded from the discussion. The area affected has been remarkably free from lightning during the last two years, as far as can be ascertained, and any serious consequence of lightning shock in previous years must surely have become apparent within this period. Lightning frequently affects tree groups irrespective of species, and incense cedar would have suffered as much as the other species from a shock severe enough to cause death.

All the trees examined show that the annual ring of 1924 had stopped its growth relatively early in the season, while their sound neighbors had completed their growth. Only part of the spring wood is formed and the summer wood, which at the time of the examination should have been completed, is entirely lacking. Generally the ring has attained not more than one-fourth of the width of the 1923 ring, which latter is not below normal in spite of the drought prevailing in that year. As far as it is permissible to guess, the 1924 increment stopped some time in June or July. It is not probable that the insects now present were at work then and that the killing showed up two months later.

All indications point to the influence of a primary agency of a physical nature affecting the whole forest but becoming injurious only in conjunction with unfavorable local conditions. Under this assumption, neither the primary agency nor the adverse local conditions alone are sufficiently harmful to bring the trees to the point of death. When they combine, their effect may prove fatal.

The unprecedented drought prevailing in 1924 must have made itself felt in the life of the forest. The normal seasonal precipitation for California is 25.43 inches. The average for 1923–24 was only 12.18 inches or less than half the normal. The corresponding figures for the nearest Weather Bureau Station, Lake Eleanor, situated 30 miles south at a little lower elevation, are 39.2 inches, based on a 13-year record, and 20.83 inches, or a little more than half the average. As a consequence of this severe drought condition, the annual elimination of older foliage, which normally takes place in the fall, began this year in June and in some cases even earlier. The deficiency in soil water was undoubtedly least felt in bottom land and in close proximity to streams, and became more and more pronounced on steeper slopes and ridges. It must have been most marked where bed rock comes close to the surface and where trees live in pockets formed by the bed rock or by large boulders. As the trees grow their roots fill out the pockets as potted plants will fill out a flower pot. They finally become root-bound, and, while in normal years this condition may simply express itself in a toning-down of growth, it becomes fatal under excessively severe drought conditions. The trees had already gone through a drought in 1923, and the deficiency in precipitation during the winter of 1923–24 accentuated whatever physiological harm had resulted from it. The ground water, instead of being brought back to normal, receded to still greater depths. An excavation on the area of the timber sale in September of 1924 showed that the soil was dust dry to a depth of more than 13 feet, so that even in deep soils the majority of the trees must have suffered from a lack of water. In shallow soil pockets the supply is likely to be cut off entirely. When a number of trees stand together in the same pocket the effect must be cumulatively severe. None of the groups observed are located in bottom land or close to running water. In many cases they appear on slopes or benches where rock outcrops are common. In others no surface indication of rock is apparent, but the rights-of-way of the nearby logging railroad reveal that the distribution of soil and rock is often of the nature described.

Figure 1 shows the root system of a yellow pine of about 36 inches D.B.H. The tree had stood on the right-of-way and the stump had been pulled and removed. Instead of spreading freely through the soil the roots are closely compacted into an irregular cube of the shape of the narrow



—*Photograph by L S Gill*

FIG. 1. Yellow pine stump with root system, root bound by confined growth in granite pocket.

pocket in which they grew. Figure 2 illustrates in detail the abnormal flattening of the roots against the granite walls of the pocket.

It is impossible to ascertain whether the particular tree shown in the figures had been dead when cut or would have succumbed later in the year. On one side only, a few roots have been able to escape out of the pocket into better soil and to provide the tree with a modicum of water. At any rate, the peculiar conformation of its root system may with propriety be used in the interpretation of the facts reported.

Rock pockets completely isolated from soil connected through capillarity with the deeper moister strata must be relatively rare. The attempted explanation of the phenomenon would account for the fact that the trees are dying in widely scattered groups, that the groups are composed of different species, and that death occurred at about the same time. While trees with normal root systems survived the drought with little injury and adjusted their water budget by prematurely eliminating surplus foliage, the abnormal drop in the water table made it increasingly difficult, and finally im-

—*Photograph by L. S. Gill*

FIG. 2. Same as Fig. 1. Detail of closely intertwining and rock-flattened roots.

possible, for pot-bound and isolated individuals or groups of trees to secure even the absolute minimum of moisture needed to maintain life.

The survival of incense cedar under conditions apparently identical with those which proved fatal to the other species remains unexplained. It may be that incense cedar adapts itself to adverse conditions, in particular to drought, even to a greater extent than has already been known.

The explanation attempted rests frankly upon surmise, supported by observational evidence. The handling of the root systems of timber ranging up to 40 and 50 inches D. B. H. and the excavation of huge granite pockets make detailed examinations prohibitive. Further observations along railroad rights-of-way and roads in the forest may furnish additional support for the view advanced.

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PHYSIOLOGICAL EVIDENCE ON THE GENETIC IDENTITY OF NATURAL AND SYNTHETIC STRAINS OF WILD EMMER[1]

OLAF S. AAMODT AND MOSES N. LEVINE

Love and Craig (12), while engaged in the study of the genetics of F_2 segregates of a cross between *Triticum vulgare* Vil. var. Early Red Chief, and *T. durum* Desf. var. Marouani in 1918, observed types which closely resembled the wild emmer or so-called "wild wheat" of Palestine, *T. dicoccum dicoccoides* Kcke., which had been rediscovered by Aaronsohn (2) in 1906. The appearance of the wild form in the progeny of a cross between common and durum varieties is of profound interest, because it suggests the possible origin of the prototype of the common wheats.

Experiments were made by Love and Craig to determine how the characters of the natural wild emmer were inherited when crossed with other types. They found that " . . . the characters of the wild wheat behave in hybridization much as do the characters of the other wheat types" (12, p. 62). Further intensive inheritance studies by these authors (13) and careful cytological investigations[2] have confirmed the striking similarity between the natural wild emmer found by Aaronsohn and the synthetic wild type produced by Love and Craig.

A third method of studying the similarities of the two wild-emmer forms has suggested itself, namely, their reaction toward physiologic forms of stem rust (*Puccinia graminis* Pers.). That parasitic organisms might serve as adequate physiologic reagents in the determination of the phylogenetic relationship and taxonomic position of certain plant and animal hosts has been known since the days of Darwin. The literature dealing with this subject has been reviewed by Vavilov (19, 20) and Dufrénoy (4). In the present case it will suffice to cite the instances where fungous parasites have been successfully used as physiologic reagents.

As long ago as 1895, Eriksson (5) found that a certain wheat-rye hybrid was immune from the brown leaf rust of rye, *Puccinia dispersa* Erikss., and susceptible to the orange leaf rust of wheat, *Puccinia triticina* Erikss., which seemed to indicate that this hybrid was more closely related to the wheat than to the rye parent.

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[1] The writers are indebted to H. H. Love, Professor of Plant Breeding, New York State College of Agriculture, Cornell University, for supplying them with the seed material of the natural and synthetic wild emmers. They wish to express their grateful appreciation also to E. C. Stakman, Head of the Section of Plant Pathology, Minnesota Agricultural Experiment Station, and Pathologist, Office of Cereal Investigations, U. S. Department of Agriculture, for valuable suggestions and helpful criticisms.

[2] The cytological studies were made by Ernest Dorsey, of Cornell University, and the results are being reported in a separate paper.

Klebahn (9, p. 140-141) mentions a case in which he discovered an error in the determination of a willow plant by inoculating it with a narrow specialized rust fungus, *Melampsora ribesii purpureae* Kleb.

Vavilov (18, 19, 20) used highly specialized, obligate parasites, such as *Erysiphe graminis tritici* March., *Puccinia triticina* Erikss., *P. graminis avenae* Erikss. and Henn., and *P. coronifera* Kleb., in an extensive genetic and systematic study of many varieties of wheat and oats. By the use of this method he succeeded in separating many varieties of these cereals into distinct races. The results were corroborated by the simultaneous use of other methods: hybridization, serum reactions, and cytologic examinations.

The effective application of parasitic organisms as physiologic reagents led Vavilov (20, p. 235) to the conclusion that, "this method can sometimes be very useful to genetists as well as to systematists, even for practical purposes of plant-breeding, especialy in giving useful suggestions, as to the possibility of crossing some species and varieties which morphologically may be quite distinct. For phylogenetical purposes this method can be used in the same way as hybridization, serum reactions, cytology, etc."

Stakman and Levine[3] were able to separate *Festuca elatior* Linn. from *F. pratensis* Huds., although the two grasses are considered as identical by some authorities, by the difference in their reactions to a strain of *Puccinia graminis phleipratensis* (Erikss. and Henn.) Stak. and Piem. *F. elatior* was absolutely immune from this rust, whereas *F. pratensis* was completely susceptible. Likewise they could distinguish *Agropyron repens* (L.) Beauv. from *A. tenerum* Vasey by their reaction to *P. graminis tritici* Erikss. and Henn.

Dufrénoy's (4) essay, based on the researches of Stakman and his co-workers (15, 11, 16), Chapman (3), Legrand (10), and others, points out that living organisms are the most delicate reagents of which we now have any knowledge or control. Dufrénoy, furthermore, brings out the fact that parasitic organisms can render evident those biochemic and biologic characters which are peculiar to each group of individuals and which are otherwise indiscernible.

Johnston and Bower (8) found that certain physiologic forms of *Puccinia graminis tritici* furnished them by the Minnesota laboratary could be used as an effective and rapid method in determining the purity of Kanred seed wheat. This method proved a valuable supplement to field inspection.

It has been demonstrated by Stakman and Levine (17) that several varieties of wheat show different degrees of resistance when inoculated with certain physiologic forms of *Puccinia graminis tritici*. These differences are consistent and constant and have been shown by various investigators (14, 6, 1, 7) to be definitely inherited genetic characters.

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[3] Unpublished results obtained by E. C. Stakman and M. N. Levine in 1915.

EXPERIMENTAL DATA

It was thought that a comparative study of the reaction of the natural wild and synthetic wild emmers to several physiologic forms of *Puccinia graminis tritici* might yield valuable information regrading the physiological similarities of these strains of wheat.

Five distinct physiologic forms were selected for the experiment: *Puccinia graminis tritici* forms 9, 19, 21, 27 and 34 Stak. and Lvne. Their action on differential varieties of *Triticum* spp. is given in a previous publication (17). In the present study Marquis and Kanred, common wheats, Einkorn, and Vernal were used as controls.

The plants studied were inoculated in the usual manner and the rust notes were taken two weeks after inoculation. The results are recorded in table 1. The types of infection are given according to the scale used by Stakman and Levine (17). In this scale 0 designates immunity; 1 and 2, resistance; 3 and 4, susceptibility; X indeterminate reaction.

TABLE 1.—*Reaction of synthetic wild and natural wild emmers to five different physiologic forms of Puccinia graminis tritici*

Physiologic forms of *P. graminis tritici*	Reaction to rust fungus					
	Differential varieties				Wild strains	
	Wheat		Einkorn	Emmer	Synthetic	Natural
	Marquis	Kanred		Vernal		
No. 9	4	0	3	4	4	4
No. 19	2	0	3	0	4	4
No. 21	4	0	1	0	4	4
No. 27	2	0	1	4	4	4
No. 34	4	4	1	0	4	4

As will be seen from the results presented in table 1, the degrees of infection produced by the five physiological forms are quite different on Marquis and Kanred wheats, Vernal emmer, and Einkorn, and yet they all produced a type 4 infection on the two wild emmers. Marquis differentiated forms 9, 21, and 34 from forms 19 and 27. Kanred, being completely susceptible to form 34 and absolutely immune from the other four forms, served as the differentiating host for form 34. Forms 9 and 19 could easily be distinguished from forms 21 and 27 by the difference in the reaction of Einkorn. Vernal served as the differential variety between forms 9 and 27 on one hand, and forms 19, 21, and 34 on the other.

CONCLUSION

The fact that the synthetic wild emmer and the natural wild emmer react alike to different physiologic forms of *P. graminis tritici* furnishes additional proof of their similarity, and supports the view of Love and Craig (12, 13) regarding the probable origin of the natural wild emmer. This experiment also demonstrates again the value of parasitic organisms as physiological reagents in phylogenetic and taxonomic studies.

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ROOT KNOT ON SUGAR CANE IN PORTO RICO

JULIUS MATZ

WITH TWO FIGURES IN THE TEXT

Since the middle of the summer of 1924 the writer has been finding nematode-infected root swellings or "root knot" of sugar cane in widely separated areas in southern Porto Rico. The exact extent and severity of this infestation is not yet determined, but it has undoubtedly caused the lack of growth in older cane as well as the death of young cane in a number of fields in several concentrated areas.

This nematode infection of cane can be detected by two distinct symptoms: first, by a deep, waxy, or golden yellow instead of the normal green coloration in the leaves of the older plants. This yellowing, which often occurs in rather indefinite wide bands, usually starts from the tips of the leaves and spreads downward to their sheaths. This color is distinct from the pale or white-yellow rather narrow bands in chlorotic leaves. Second, in young cane three or four months old the outermost leaves become shriveled and streaked longitudinally with red or copper colored areas divided by long, dry strips, and the whole plant becomes stunted and takes on a bunched or small-broom like appearance. Cane plants showing these symptoms when pulled up were found to have in the newer portions of their roots, mostly at the root tips, visible swellings or galls of about twice the original diameter of the root (Fig. 1). These swellings were enlargements of the outer fleshy layers only, while the central woody parts were not deformed. Nematodes are embedded in these swellings. In the older, less conspicuous galls which occur throughout the length of the roots are found the impregnated female nematodes. These females are about 1 mm. in length, milky white, glistening, usually in the form of a diminutive bulb with the extended thin neck and head distinguishable from the rest of the egg-full body (Fig. 2, a). As the outer fleshy layer of the cane roots ultimately shrinks and becomes spongy and brown, the galls become greatly reduced. In this condition by bending the root and thus breaking the epidermis, the larger females are exposed and found situated in rounded cavities. These cavities become larger after the disappearance of the inhabiting nematodes, owing to the shrinking of the surrounding spongy tissues (Fig. 2, b).

The main apparent physical injury which this nematode causes is the small cavities in root tips which are finally destroyed. A general decay of the roots does not usually occur, although they may become riddled with

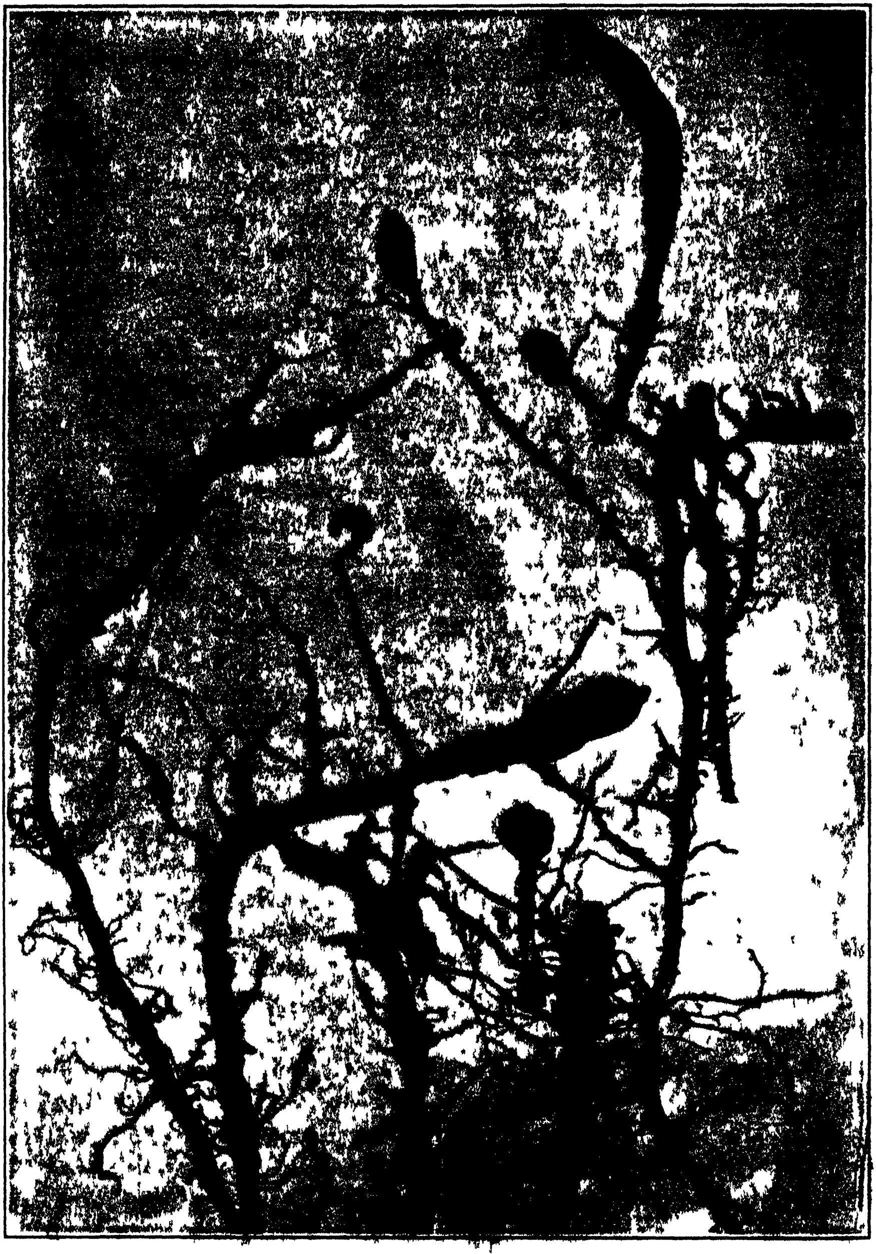

FIG. 1. "Root Knot" on sugar cane roots. ×2.

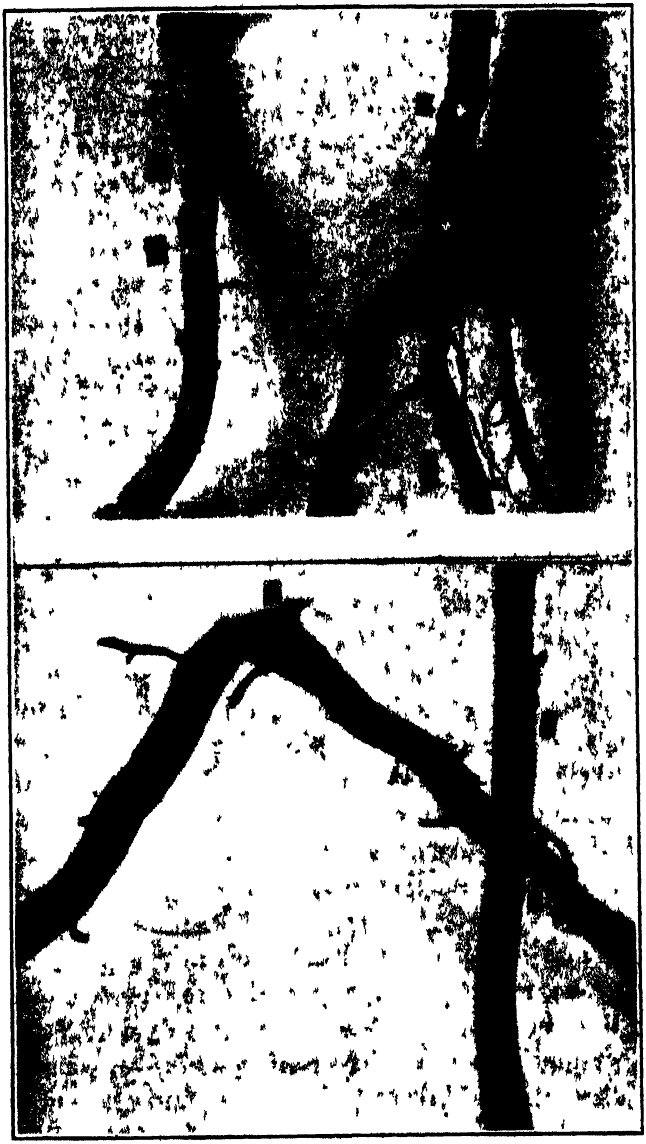

FIG. 2. Sugar cane roots infected with nematodes. *a*, the white globular but pointed females full of eggs are exposed after tearing away the epidermis of the root; *b*, empty old nematode cavities in roots. ×3.

holes, but root-tip decay was quite commonly seen in association with nematode infestation. Sometimes, however, many new rootlets grow out from near a gall, but the newer roots in turn may also become infested. The effect on the growth of the cane is very marked when nematodes are abundant. The cane leaves become yellow, and there is a very evident retardation of growth of the whole plant. The death of young cane due to severe nematode injury has been seen in three fields.

Nematode infestations of sugar cane have been found only in loose or sandy soil, or in land with a loose or sandy subsoil.

Krüger (2) says that Prins, Treub, Saltwedel, and he have observed two types of nematodes, *Tylenchus sacchari* Saltwedel, and *Heterodera radicicola* Müller, on sugar-cane roots in Java, between the years 1885–1887. Tylenchus apparently did not produce swellings in the roots, while Heterodera, quoting Dr. Krüger, "builds on the roots of sugar cane up to hazel-nut size galls" (Translation). Neither of these two species would coincide with the rather small gall-producing nematode in Porto Rico. Because of the gall-forming habit of this nematode, it is also placed in the genus *Heterodera*.

Cobb (1) relates how he found a Tylenchus on the roots of sugar cane in Hawaii in 1907. He does not mention the appearance of galls or swellings in the attacked roots.

Rands (3) described the damage caused by pitting of the terminal portion of sugar-cane roots in Louisiana by a small snail (*Zonitoides arboreus* Say). These cavities or pits were 0.3–2.0 mm. in depth and diameter. They were similar to the cavities made by nematodes in the roots of sugar cane in Porto Rico, where no snails were associated with the pitted roots, but where nematodes were found in the unexposed cavities. Rands does not mention the occurrence of swellings or galls associated with snail injury.

The symptoms of nematode injury are distinct and peculiar to this affection of cane roots. While a partial decay of the roots is caused directly by these nematodes, root knot becomes only an added phenomenon to, rather than an explanation of, root diseases of sugar cane in Porto Rico. Other root diseases have their own distinctive symptoms and causes, as has been shown in previous publications. Apart from nematode injury and root infection by *Plasmodiophora vascularum* Matz there is no clearly defined cane-root disease on the irrigated south coast lands of Porto Rico.

This root knot on sugar cane occurs on loose, though sometimes wet, more or less sandy soils on varieties, Cristalina, BH 10 (12), and SC 12/4. It has not been seen on the heavier, moisture-retaining silt or clay soils. According to the observations of the Javanese investigators, however, moisture seems to favor nematode occurrence on sugar cane in Java. While on high land, after a prolonged dry period, living nematodes were rather rare there.

The bad effects of the nematodes were overcome in one field of young cane growing on a porous soil consisting of about an 8-inch top black layer underlaid with several feet of brown sand and coarse gravel by applying a layer of barnyard manure to the roots of the cane and then covering this with soil, after which the field was kept moist by frequent irrigation. This gave the cane plants an opportunity to develop a new root system in a new and fertile layer of soil. This seems to be a plausible means of control as the nematodes seem to be most abundant and are at least more injurious in soils where there is less stimulus to root growth.

FORTUNA,
PORTO RICO.

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OAT BLAST

CHARLOTTE ELLIOTT

In oats throughout the United States there is present each year a condition of some of the spikelets which in this country has been designated as "blast" or "sterility" and in England as "blindness," "deafness," or "white ear." These blasted or undeveloped spikelets usually are white and range from minute rudiments to fairly well developed empty glumes. Occasionally the larger empty glumes contain chlorophyll. The numbers of blasted spikelets in different fields, and under different environmental conditions, vary considerably. At a conservative estimate they may in some cases amount to 40 per cent of the total number. In North America this condition of the spikelets has been attributed to thrips (2) and to the bacterial disease known as "halo blight" (3). In England frit flies were found associated with the blasted spikelets and considered as the cause (4).

The results of observations and experiments in Wisconsin during 1918 and 1920 (1) showed conclusively that, while the bacterial disease known as halo blight is in some cases associated with blast, on the whole there is no connection between the two, blasted spikelets appearing quite independently and in fields where the bacterial disease does not occur. The results of this work also indicate that environmental conditions may be important factors in the production of blast. To determine the possibility of a correlation between temperature and rainfall at heading time on the one hand and blast on the other and to learn whether or not there are rather constant varietal differences in blast from year to year, counts have been made for the past three years on a number of varieties of winter oats grown in the varietal experiments conducted by the Office of Cereal Investigations at Arlington Farm, near Rosslyn, Va., under the direction of T. R. Stanton and J. W. Taylor. All spikelets were counted as blasted in which no kernel developed The results of this preliminary work are summarized in table 1.

The varieties listed in the tables were grown in fortieth-acre plats. On 24 panicles selected at random in each plat counts were made of the number of normal and blasted spikelets. For most of the varieties the plats were replicated several times, hence the counts were correspondingly repeated. Thus the number of panicles counted amounted to from 2 to 4 times the twenty-four for each plat. Averages of the entire number in each variety were taken and percentages of blast determined.

TABLE 1—*Average percentages of blast in winter oat varieties grown at Arlington Farm, Virginia, in 1922–24*

1922			1923			1924		
Variety	C. I.[a] No.	Pct. blast	Variety	C. I. No.	Pct. blast	Variety	Pct. blast	C. I. No.
Hatchett	838	6.38	Hutcheson Selection	947	17.77	Fulghum	708	11.54
Kanota	839	6.84	Fulghum	708	19.57	Kanota	839	15.61
Fulghum	708	8.98	Kanota	839	21.79	Hatchett	838	21.64
(Black)[b]	691	13.47	(Black)[b]	691	25.45	(Black)[b]	691	22.08
Hutcheson Selection	947	15.67	Winter Turf	541–4	26.13	Hutcheson Selection	947	25.58
Culberson	273	16.16	Red Rustproof	1815	31.05	Aurora	831	27.54
Dwarf Culberson	748	17.43	Culberson	273	32.79	Culberson	273	27.92
Aurora	831	20.61	Winter Turf	435–4	33.39	Dwarf Culberson	748	28.66
Winter Turf	541–4	22.05	Winter Turf	431	34.21	Bicknell	206–155	29.22
Red Rustproof	1815	23.93	Bicknell	206–155	34.51	Winter Turf	541–4	30.29
Winter Turf	435–4	24.84	Dwarf Culberson	748	34.95	Red Rustproof	1815	31.52
Winter Turf	431	26.52	Lee	2142	37.00	Winter Turf	431	32.53
Bicknell	206–155	27.65	Aurora	831	37.48	Winter Turf	435–4	32.64
Ferguson Navarro	966	28.00	Custis	2141	40.49	Lee	2142	38.23
			Ferguson Navarro	966	46.62	Custis	2141	38.47
						Ferguson Navarro	966	45.47

[a] Cereal Investigations accession number.

[b] An unnamed black strain similar to Hutcheson Selection.

The varieties are arranged in the order of increasing percentages of blasted spikelets. A comparison of the data available for the three years brings out three facts which throw some light on the problem of blast in oats.

First. Varietal differences in the amount of blast appear to be fairly constant from season to season. The five varieties, Hatchett, Kanota, Fulghum, Hutcheson Sel., and (Black) C. I. No. 691, show the lowest percentages throughout the three years. Culberson and Dwarf Culberson are intermediate, and Ferguson Navarro, Lee, and Custis consistently show high percentages of blast. The greater variability of some of the varieties may or may not be due to the limited data.

Second. Percentages of blast in all varieties vary from year to year, all percentages being higher in some seasons and consistently lower in others. In 1922, all of the varieties listed showed comparatively low percentages of blast. In 1923 the percentages throughout the list are much higher—in some varieties more than double those of the previous year. In 1924 the percentages are intermediate with the exception of 4 varieties, Hutcheson, Red Rustproof, Winter Turf 541–4, and Lee. The decided contrast in percentages of blast in 1922 and 1923 seem to indicate that certain seasons are much more favorable for the development of blast than other seasons, and that environmental conditions are possible factors in bringing about these differences. As the oats were grown on the same farm and on practically the same type of soil throughout, this factor may for the present be eliminated. In attempting to correlate the high percentages of blast with rainfall and temperature at the time of heading, the following figures are of interest. The ranges in the percentages of blast for the three years are respectively: 6–28%, 17–46%, 11–45%. The total precipitation for May of each year, the month when the plants head out, was 4.53 inches, 1.49 inches, and 6.59 inches respectively. It is at once evident that in 1923, when percentages of blast were highest, the rainfall at heading time, instead of being above normal was much lower than in either 1922 or 1924 and considerably below the normal of 3.[illegible] inches. Furthermore the exact precipitation dates in 1923 do not coincide with dates of heading of the different varieties. In 1923 all varieties, with the exception of Winter Turf, headed during a period of no rainfall. In 1924 most of the varieties headed at a time when there was some rainfall nearly every day. Weather reports for 1922 and 1923 show that the blast of 1923 is not connected with drought conditions during earlier months, for precipitation was near or above normal in the preceding months. Variations in temperature were greater in May, 1923 and 1924, than in 1922, but for the present no attempt is made to connect these wider ranges in temperature with the greater number of blasted spikelets.

Third. The varieties which show the highest percentages of blast, i.e., Ferguson Navarro, Custis, and Lee, are either of known or suspected hybrid origin. Lee and Custis are new varieties developed from a hybrid of Aurora × Winter Turf by T. R. Stanton. According to Mr. Stanton there is some reason for thinking that Ferguson Navarro also is of hybrid origin as it shows some characteristics of both red and common oat varieties. It originated as a stray plant in a field of Red Rustproof oats in Navarro County, Texas.

What is known regarding the sterility which follows certain crosses both in plants and animals may have some bearing on the high percentages of undeveloped spikelets in the hybrid varieties Lee and Custis and also in Ferguson Navarro.

Work along similar lines is in progress.

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PHYTOPATHOLOGICAL NOTES

Polyporus schweinitzii Fr. on Douglas Fir in the Eastern United States. —Very severe losses from cankers attributed to *Phomopsis pseudotsugae* Wilson have been reported in Scotland in plantations of Douglas fir, *Pseudotsuga taxifolia* (Lam.) Britt. Since trees of this species had been shipped to the United States among the many importations of conifers from Great Britain before the present quarantine laws went into effect, it is feared that this disease may have been introduced in this manner. The Office of Investigations in Forest Pathology, as opportunity offers, is examining all plantations of Douglas fir for this disease.

One of the small plantations of this species inspected August 19, 1924, is located on the Vanderbilt estate near Biltmore, North Carolina. The trees of Douglas fir in this particular area were four-year-old transplants when set out in 1896 by Dr. C. A. Schenck. The *Phomopsis* canker was not found at the time of inspection but it was noted that the red-brown butt and root-rot due to *Polyporus schweinitzii* Fr. was present and causing considerable damage. Six trees were found wind-thrown in a slight depression on a northwestern slope, despite a cover of planted white pine surrounding the plantation. The fallen trees ranged from 18 to 24 feet in height and from 3½ to 5 inches D. B. H. The butts and roots, especially the main tap roots, were badly rotted and greatly weakened by *Polyporus schweinitzii*. At the base of each of two of the wind-thrown trees which were still alive, typical sporophores of this fungus were found in direct association with the red-brown rot, indicating that it was the cause of the decay.

This plantation of Douglas fir has not thrived. Although it has not been thinned or given much care, and some of the trees have apparently been cut for Christmas greens, the primary cause of failure is very probably the attack of *Polyporus schweinitzii*. This fungus has never been reported seriously injuring young Douglas fir trees in the northwestern United States, although it is very destructive to older ones there. This indicates either a greater virulence in the strain of the fungus present or a greater susceptibility of the host tree in the locality near Biltmore.

Polyporus schweinitzii is known to attack various species of pine, spruce, fir, and hemlock in the eastern United States, and in this instance the fungus probably spread from trees of some of these species to those of the Douglas fir. This fungus has been reported as attacking young Douglas fir in Great Britain, and is frequently found in other conifers in Europe. It is possible that introduced species by a change of habitat may be rendered more susceptible to root rots than trees in the native stands.—G. G. HEDGCOCK, G. F. GRAVATT, AND R. P. MARSHALL, OFFICE OF INVESTIGATIONS IN FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY, WASHINGTON, D. C.

Fire at Clemson College. The Agricultural Hall at Clemson College burned early in April. The Division of Botany and Bacteriology was on the first floor and suffered heavy losses. The following members of the personnel of the Division lost their files of reprints and would appreciate receiving complete sets of separates: D. B. Rosenkrans, pathology, physiology, and forestry; Mr. and Mrs. W. B. Aull, bacteriology and general botany; W. D. Moore, pathology (reprints received more than a year ago were saved); L. M. Fenner, pathology; and C. A. Ludwig, pathology and physiology. The Experiment Station library also was destroyed, and the librarian, Mrs. Crown Torrence, would be pleased to receive separates and bulletins.—C. A. LUDWIG, CLEMSON COLLEGE, SOUTH CAROLINA.

BOOK REVIEWS

MANUAL OF VEGETABLE-GARDEN DISEASES. By C. C. Chupp, Ph.D. New York. The Macmillan Co., 1925, pp. 647, figs. 155. Price, $4.00.

In 1874 Sorauer's classic work entitled, "Handbuch der Pflanzenkrankheiten," was published. Twenty-two and twenty-three years later, "Pilzpärasitaren Krankheiten der Pflanzen," and "Diseases of Plants Induced by Cryptogamic Parasites," by Frank and by von Tubeuf and Smith, respectively, made their appearance. These efforts, while good in the light of the knowledge of plant diseases at that time, were soon out-of-date. Some years after the appearance of the works by Sorauer, Frank, and von Tubeuf, Americans entered the field. As an outgrowth of the development of the science in the United States, a number of books, texts as well as handbooks of reference, attempting to cover the diseases of crops as a whole or in part, have appeared. Most of these books have not quite come up to anticipation either from the standpoint of a reference for research workers or from the standpoint of those uninitiated into the realm of phytopathology. Chupp, with a book of 647 pages, entitled "Manual of Vegetable-Garden Diseases," is the last to enter the field. The purpose of the book as implied in the language of the author is to bring "together all the material in such a form that it is available to the plant pathologists generally and to the growers and extension specialists in particular." To bring together *all* the material in an available form is no small undertaking. No regional territorial limitations are claimed for the book, from which it will be inferred to be of world-wide application.

The book is attractively bound, printed with a clear type on good paper, and presents an attractive appearance. The entire subject-matter is divided into twenty chapters. Each of the first eighteen chapters treats of the specific diseases of a crop or group of crops generally related. The nineteenth chapter discusses the various phases of soil sterilization, and the twentieth treats of the use and application of fungicides. An entire chapter is given to the diseases of the more important crops or to the crops whose diseases are best known. This mode of procedure, however, is not always followed, since chapter three is given to the diseases of beets, carrots, and chicory; chapter eleven to okra, parsley, and parsnip; and chapter fifteen to rhubarb and salsify. These combinations are apparently made wholly on the grounds of their alphabetical order, notwithstanding the fact that a better combination could have been made on the basis of common diseases, as, for example, chicory and lettuce. As a matter of fact, it would probably have been better, both from the standpoint of consistency and clearness, to have given an entire chapter to each crop, even though it is a relatively unimportant one and the diseases few.

Each disease, if an important one, is discussed under symptoms, cause, and control. This arrangement of subject-matter is good and is helpful to the reader in finding the particular features of the disease in which he is interested.

There are 155 illustrations reproduced as text figures. Many of these illustrations are typical of the disease as a whole and are well reproduced. Others, on the other hand, are not typical or are poorly reproduced. It is to be regretted that the author copied so many of the illustrations from bulletins and journal articles. In many cases copies were made when high-grade prints or even negatives could have been secured or borrowed.

The author shows a lack of familiarity with the diseases of southern crops. One looks in vain for any mention of cassava, chayote, yam, udo, and dasheen, all of which are grown in the United States and some of which are becoming important food crops. If, as claimed by the author, the book is to be of particular use to the extension specialist, it would be highly desirable to have information on the diseases of these crops, if any such occur, made available for him in a manual of this sort. Furthermore, if diseases of certain vegetable crops are unknown or have not been studied, the extension specialist would welcome such information.

The author has discussed in more detail than would appear necessary the well-known diseases of the major crops. One hundred and fifteen pages are devoted to the potato alone, thirteen pages to a discussion of *Rhizoctonia*, and eight to late blight. These diseases, as well as potato scab, are well known, even to the majority of farmers where they occur. It would seem that a briefer discussion of such dseases would have sufficed, thus giving space for the inclusion of crops that have been omitted.

As a substitution for the omissions mentioned above, one finds included a discussion of some, but not all diseases, which have been reported only in foreign countries, as, for example, Zopfia root-canker of asparagus, caused by *Zopfia rhizophila*. Leptothyrium canker of asparagus caused by *Leptothyrium asparagi*, Phoma root-rot of carrot caused by *Phoma sanguinolenta*, Corynespora leafspot of cucurbits caused by *Corynespora melonis*, Phytophthora fruit-spot of eggplant caused by *Phytophthora melongenae*, and black dot-rot of eggplant caused by *Rhabdospora melongenae*.

Some diseases of important crops in the United States have not been mentioned, as, for example, *Rhizoctonia* and root-knot of sweet potatoes, damping-off of eggplant caused by *Pythium*, and hollow stem of tomatoes. Some of these diseases are of more economic importance and more destructive than some of those mentioned.

The book for a first edition is relatively free from typographical errors and misstatements. A few have been noted. On page 38, line 8, we find "ascerigerous"; on p. 38, line 11, "chalamydospores"; on p. 43, line 13, "open sori or short stalks"; on p. 630, line 1, "perthecium"; on p. 58, line 34, "yars" for "years," and on p. 379, line 36, "Weimar" for "Weimer."

Each chapter is closed by the citations of literature. This is a commendable feature of the book. The literature cited has for the most part been carefully selected from the more reliable sources and from the more recent publications. This is particularly noteworthy, since the method of handling citations will facilitate acquaintance with original or more detailed treatment of subjects involved.

Some unfortunate mistakes have been made in the citing of references. For example, on p. 524, line 2, we find "Phytopath." which should read, "Jour. Agr. Research." On p. 398, line 36, "The regeneration of first generation hybrid potatoes to the wart disease" should read "The reaction of first generation hybrid potatoes to the wart disease"; and on p. 536, line 3, "Effect of copper soap spray mixtures on control of tomato leaf-spot" should read "Effect of copper soap and of bordeaux soap spray mixtures on control of tomato leaf spot."

The book is undoubtedly the best that has yet been produced in this particular field. In that there is brought together for ready reference information relative to the identification and control of the more common vegetable diseases, the book is one which will be useful to extension workers as well as to the investigator. From the standpoint of the research worker at least, the author often speaks with more positiveness and finality than the facts in the case will warrant; likewise, deductions are sometimes made which from the information contained in the literature hardly seem justified

All in all, Dr. Chupp is to be commended for having acquitted himself so well at such a difficult task. He has not only demonstrated a first-hand knowledge and familiarity with many of the garden crop diseases, but he has combined this information with that contained in a mass of literature to produce a book which is not only better than any book of its kind yet published but which is really good. It is to be hoped that the book will receive a careful re-editing before another edition is printed.

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PHYTOPATHOLOGY

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CONDITIONS ANTECEDENT TO THE INFECTION OF WHITE PINES BY CRONARTIUM RIBICOLA IN THE NORTHEASTERN UNITED STATES

PERLEY SPAULDING AND ANNIE RATHBUN-GRAVATT

WITH FIVE FIGURES IN THE TEXT

Investigations of *Cronartium ribicola* Fischer by the writers during the past few years have yielded a number of random notes on the conditions antecedent to the infection of white pines by this fungus in the Northeastern United States. As none of these observations fitted into any other article prepared for publication, they have been collected in the present one.

FACTORS INFLUENCING THE PERIOD OF PRODUCTION OF THE TELIA

In 1922 Spaulding (6) published the available data on the time of the formation of the first telia of *Cronartium ribicola* in infected areas of the Northeastern United States and Europe. These dates may be, and usually are, quite different from those for the formation of telia in abundance in the same localities, as it commonly takes about two weeks or more for their abundant development to take place. For example, near Warrensburg, New York, in 1923, telia were not abundant until the latter part of July, and in 1924, not until the first of August. Yet, in both years the first telia were formed about July 4. While the weather conditions somewhat retard or accelerate the formation of the first telia, they seem to have a more potent influence upon the formation of the telia in abundance. The season of the production of the telia lasts from the formation of the first ones until the fall of the leaves of their *Ribes* hosts. This latter date varies with the species of *Ribes* and the weather conditions of the latter part of the season. Drought in August or later is quite sure to cause the fall of practically all of the leaves of such species as *R. cynosbati* (L.) Mill., *R. glandulosum* Grauer, and *R. rotundifolium* Michx. The premature fall of infected leaves is hastened further by the action of the blister rust fungus. Also the *Ribes* species vary in the time of season when they normally drop their leaves; some ripen them and prepare for winter rest earlier than others. *R. cynosbati, R. glandulosum,* and *R. rotundifolium* are not only especially affected by drought and by blister rust attacks but they also, together with *R. vulgare* Lam., normally shed their leaves early. *R. nigrum* L. and *R. odoratum* Wendl. are examples of species which retain their leaves for a maximum time. Species of *Ribes* also differ much in their ability to continue producing new leaves throughout the season, and in their ability to produce a second crop of leaves after premature defoliation. *R. glandulosum* does not commonly form a second crop of leaves after defoliation, while *R. nigrum* usually continues to form new leaves throughout the season. The other northeastern species of *Ribes* are intermediate between these two in their leaf-producing ability.

FACTORS INFLUENCING THE GERMINATION OF TELIOSPORES

Newly matured teliospores of *Cronartium ribicola* appear to germinate with equal vigor regardless of the species of *Ribes* upon which they are produced. Practically every teliospore of newly and fully matured telial columns has been seen to germinate naturally when floating upon the surface of water. In the northeastern United States it is probable that comparatively few teliospores fail to produce sporidia sometime before they lose their viability. The climate in those regions where this fungus is now spreading naturally is such that moisture in some form apparently reaches most of the telia in sufficient quantities to cause the teliospores to germinate before they lose viability. Moisture seems to be the prime requisite for the germination of the teliospores. Temperature *per se* does not seem so important in limiting their germination. Low temperatures are definitely known not to prevent germination, as teliospores have been found profusely germinating upon leaves lying upon melting snow (7). Moreover, they have been germinated by the writers at out-door temperatures from August 1 to November 8 (7, 8). No test has been made of the effect of high temperatures upon germination of the teliospores, although Doran's (4) tests with the aeciospores and urediniospores of this fungus would indicate that the higher summer temperatures may prevent germination even in the presence of water. The effect of high summer temperatures may be due more to the accompanying dryness than to the heat alone. Definite tests are needed to determine the facts.

The oldest teliospores in a telial column are located at its tip, while the youngest ones are at its base (3, 11). The oldest teliospores germinate first. Immature columns are shorter than mature ones. When immature columns were placed under conditions favorable for germination in our cultures, only the teliospores at their tips germinated. This reaction has been well illustrated by Tulasne (11) for *Cronartium asclepiadeum* Willd., in one of the earliest figures ever published of germinating teliospores of a *Cronar-*

tium. The length and diameter of the mature telial columns of *C. ribicola* vary with the host species upon which they are borne (6, 10). Those borne upon leaves of *Ribes glandulosum* are longer and more slender than those upon most of the other species of *Ribes* found in the Northeastern United States.

Each cell of a telial column (each teliospore) germinates independently of the rest. Physiologically it is as much a separate entity as though it actually were separate morphologically, as, for instance, the aeciospores or urediniospores of this fungus. After a teliospore has completely germinated, nothing is left of the original spore except an empty shell. When the tip teliospores of a column have germinated, the empty cells fall apart readily and are commonly infested by numerous saprophytic fungi such as *Alternaria,* etc. The exhausted cells appear to be much more readily penetrated by water than are the ungerminated ones. While some of the inner teliospores of a column undoubtedly germinate when the outermost ones do (3) it is a question whether many of them do so until the outer ones have germinated and their empty shells have been sloughed off. Reed and Crabill (5) found that this happens with the teliospores of *Gymnosporangium juniperi-virginianae* Schw., which are borne in elongate protuberances somewhat similar to the columns of *Cronartium ribicola.* When inner teliospores of *C. ribicola* do germinate with the outer ones, they are able to push their germ tubes between the overlying ones and form their promycelia on the outside of the column. This undoubtedly is much more easily done when the outer cells are empty and fall apart more readily.

The time required for the germination of teliospores varies considerably with conditions. York[1] found that they germinated readily and copiously at 75° F. in six hours, if tested when fresh and if they are not formed in droughty weather. General experience has shown that they are injured by drought if it occurs at the time of, or immediately after, their formation. The writers found that, at temperatures of 55–70° F., fresh teliospores germinated and formed sporidia in maximum numbers in about 12 hours (8). If the telia-bearing *Ribes* leaves were kept air-dried and lying loose in papers as botanical specimens are usually kept, numerous tests showed that the time for germination and the production of sporidia increased directly with the increase in time of storage. Repeated weekly tests showed that this was also true for teliospores kept out-of-doors exposed to wind and rain (8).

The number of telia, and consequently of teliospores and of sporidia, produced by a given unit of leaf area varies greatly with the different species of *Ribes,* as was shown by Taylor (10). In this respect *R. nigrum* far surpasses all other species of *Ribes* found in the Northeastern United

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[1] Communicated orally.

States. This, rather than greater viability of sporidia produced upon it, may explain why this species causes relatively heavy infection of pines. The number of sporidia produced at a given source directly controls their abundance at given distances from this source, as can be shown by well known mathematical formulae. The frequency of their occurrence at a given distance from their source has a direct effect upon the number of pine infections caused by them. This is shown by the fact that frequency of pine infections decreases with increase in distance from the source of sporidia.

FACTORS INFLUENCING THE LONGEVITY OF TELIOSPORES

The length of time a given collection of telial columns continues to contain germinable teliospores can be tested in two ways: (1) by storing the columns in an air dry condition, or (2) by storing them out-of-doors under conditions which simulate the natural ones. Telial columns should be tested for germination at frequent intervals by "floating" on water or by placing in moist chambers (7, 8). The first method of storing gives a simple test of loss of viability while the second method involves both a test of loss of viability and a test of the rate at which the teliospores germinate under natural conditions. In the latter method the effects of the two factors can not be separated. In nature the telial columns would be subjected to conditions which approximate those of the second method of storage. In this paper "longevity" is expressed in terms of time during which there remained ungerminated and still viable teliospores. When stored out-of-doors in mosquito netting bags, the longevity of different collections of teliospores varied from about 3 weeks to about 3 months (7, 8). Under these conditions the longevity of teliospores on naturally fallen leaves collected from the ground was approximately the same as that of those on leaves collected at the same time from *Ribes* bushes. The casting of infected *Ribes* leaves did not shorten the longevity of the teliospores when they were stored under artificial conditions, and apparently did not decrease the number of sporidia produced by them. As a matter of fact, the teliospores on leaves lying on the surface of the soil would be in a more favorable position for the production of sporidia than those on leaves still attached to the bush, because moisture might reach the telia more readily on the soil. This is especially true in a time of drought, as the fallen leaves would be more easily moistened by dew. In the case of a swamp-inhabiting *Ribes*, the fallen leaves would be moist at times when the leaves on the bushes were still dry. However, the length of time that they would bear germinable teliospores would be shortened by the conditions favoring early germination and exhaustion of the teliospores.

An important factor controlling the longevity of teliospores upon leaves killed by frost or falling to the ground is the character of the leaf upon which they are borne. This was observed distinctly with the material exposed by us to the weather in our tests of the longevity of teliospores (7, 8). The leaves of *R. nigrum* remained firm and in good condition the longest of any of the eight species which were tested. Those of *R. odoratum* and *R. americanum* Mill. were nearly as weather resistant as those of *R. nigrum.* Those of *R. rotundifolium* became very brittle and tended to break into pieces. Those of *R. cynosbati* were so soft in texture that they matted together when wet, and soon decayed, thus causing the teliospores to germinate early. Those of *R. triste* Pallas acted much like those of *R. cynosbati.* Those of *R. glandulosum* tended to fall to pieces from weathering, and matted into a damp mass in which the teliospores soon became exhausted by germinating. This effect was retarded in our tests by shaking the leaves up after each rain (8).

The habitat of the *Ribes* host also has a direct influence upon the longevity of the teliospores upon its leaves. Some species, such as *R. glandulosum,* typically grow in wet situations, where the fallen leaves soon mat down and usually remain damp, so that the teliospores are soon germinated. Others, such as *R. cynosbati,* flourish in dry locations, where the fallen leaves, in spite of their soft texture and rather fragile character, resist decay for a long time. This may help to explain why damage to white pines from the latter species is usually high, while that from *R. glandulosum* is apt to be low. The fact that the leaves of *R. cynosbati* usually remain dry longer than those of *R. glandulosum* favors their blowing around and thus disseminating the disease.

DISCUSSION OF WEATHER CONDITIONS IN RELATION TO PINE INFECTIONS

York and Snell (12) found that infection of one- and three-year-old seedlings of *Pinus strobus* L. may occur within 12½ hours after inoculation with sporidia of *Cronartium ribicola.* They also found that sporidia can begin to germinate one hour after maturing. Allowing 5 hours, the minimum time found by them to be necessary for the formation of mature sporidia, a period of 18 hours of suitable weather would be sufficient for some infection of pines to take place (12). Many times it would take some hours longer for abundant infection to occur, as the writers got abundant crops of sporidia only after 12–20 hours (9). Work with the sporidia (9) shows that when the atmosphere contains more than 70 per cent relative humidity, they will survive an exposure in an air dried condition for as long as 26 hours. From August 1 to October 10, 1923, there were at Warrensburg, New York, seven periods, which were 36 or more hours long, during which the relative humidity of the air remained above 70 per cent. It appears, then, that infection of pines might have taken place during any or all of these apparently favorable periods.

Prolonged experience shows that the teliospores of *Cronartium ribicola* need moisture for their germination. Rain, dew, or heavy fog seems to be needed to cause them to germinate freely under natural conditions and to produce an abundant crop of sporidia. In fact, they are frequently found germinating and producing sporidia in nature immediately after a rain, especially if the rain is followed by cloudy weather. The sporidia are undoubtedly largely wind disseminated. It is known that the sporidia of many of the Uredinales are forcibly thrown off when they are mature (1), and it is assumed that those of this fungus also are thus set free. The slightest air current will carry them away. There probably is not an instant when slight convection currents are not in motion and capable of carrying these extremely light bodies to an indefinite distance. Falling precipitation is known to carry down minute particles such as dust, bacteria, and fungous spores which float in the air, the amount of such material in the air being at a minimum just after a rain or snow storm (2). Therefore there can be no effective dissemination of the sporidia while rain is falling. If a rain continues so long that most of the teliospores germinate and form sporidia, the latter, which are normally set free upon reaching maturity, are carried down by the falling drops of rain or mist. It appears, then, that natural infection of pines must take place during cloudy weather following a rain, fog, or dew, which is of such a length that the teliospores germinate freely and form a maximum crop of sporidia. The rain must not continue much longer or most of the sporidia will be washed down from the air as soon as formed, and thus rendered innocuous to the pines. It is not likely that sporidia, once washed to the ground, are ever again picked up in a viable condition by the wind. Sunny weather dries the air to such a point that the sporidia can not survive any length of time. The amount of air moisture is reduced quite promptly in full sunshine. The resulting dryness stops germination of teliospores and the rapidity with which sporidia are formed and set free.

While the knowledge about all of the factors concerned in the infection of pines by sporidia of *Cronartium ribicola* is admitted to be very incomplete, it seems that enough is known to justify an attempt to express their relation graphically. Figures 1 to 5 are intended to indicate certain conditions, part of which presumably result in infection of pines, and part of which presumably result in no infections. They are merely suggestive and represent but a few out of many combinations of conditions which might have been used. They are based upon data obtained in actual field work. The periods of time indicated undoubtedly will change with accompanying conditions, and in some instances they will be shortened. The present known minimum periods of time are used. The spaces from left to right indicate hours. The various bars are so designated as to be readily interpreted.

Figure 1 is intended to show conditions presumably favorable for rather heavy infection of pines. The upper bar represents the occurrence of rain, cloudy weather and sunshine during the indicated periods. Rain for 7 hours, ending quite abruptly, is followed by a period of 23 hours of cloudy weather and night; after 30 hours sunshine appears and continues. The solid black part of this bar represents rain, the stippled part cloudy weather or night, and the unshaded part sunny weather. The bar next to the top indicates the period of production of the sporidia by the germinating teliospores. This starts at the end of five hours, the known minimum (12) from which infection has been induced. In a single test by the writers (9), sporidia began to form in three hours, but this appears to be earlier than usual. The five hour period is based on numerous tests, and is therefore adopted here. The production of sporidia ends when the sun appears, after thirty hours. The middle bar shows the period of dissemination of the sporidia. The first part of it is dotted to indicate very limited dissemination while rain is falling. Promptly upon the termination of the rain, dissemination is in full swing, as it depends upon production which is shown to be heavy at this time. Dissemination ends with the stoppage of production, when the sunshine begins. The next to the last bar represents the germination of sporidia. This is known to start within one hour after they mature (9, 12). It also stops with the beginning of the sunshine. The last bar indicates the period of infection of white pines. The known minimum period for this is 11½ hours after germinating sporidia are placed upon the leaves of the pines (12). It is assumed that about one hour is needed for germination to attain a maximum rate. Therefore this bar starts 17½ hours after rain began (11½ after the first germination of sporidia) and continues until the sun shines. As far as external conditions are concerned, it appears that this set of conditions ought to result in rather heavy infection of neighboring white pines. But it must be stated that we know noth-

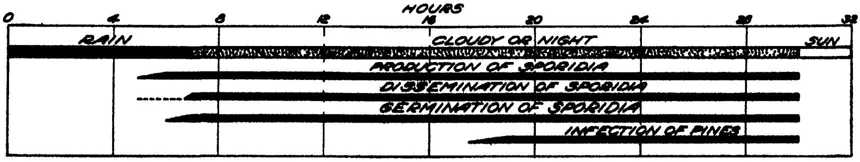

FIGURE 1. Bar diagram showing weather conditions presumably favorable for the heavy infection of *Pinus strobus* by the sporidia of *Cronartium ribicola*. The top bar represents the weather conditions. The solid black portion of it indicates rain; the stippled portion, cloudy weather or night; and the unshaded portion, sunshine. The second bar represents the period of production of sporidia. The third bar represents the period of dissemination of sporidia. The fourth bar represents the period of germination of sporidia. The fifth bar represents the period of infection of pines. This diagram presupposes a large crop of recently matured telial columns.

ing of the conditions within the pine leaves which favor or inhibit infection. A study of the infections of white pines which have occurred naturally in the forests shows plainly that heavy pine infections occur only once in several years, the intervening years being periods of mere scattering infections. It is quite evident that all periods of the type shown in figure 1 do not result in heavy infection of pines; otherwise there would be no coping with the disease before it destroyed our white pine stands. There is scarcely a year during which there is not one or more periods of weather similar to that sketched above. There is some unknown factor which has a decisive influence in the infection of white pines.

Figure 2 shows conditions which will presumably result in slight infection of pines. Conditions are the same as those shown in figure 1 except that the sun begins to shine earlier (soon after infection has started). It is quite evident that the total amount of infection would be small.

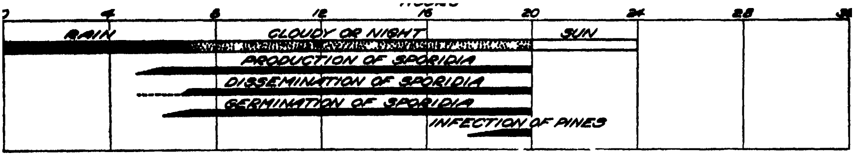

FIGURE 2. Bar diagram showing weather conditions which would presumably result in slight infection of *Pinus strobus* by the sporidia of *Cronartium ribicola*. The top bar represents the weather conditions. The second bar represents the period of production of sporidia. The third bar represents the period of dissemination of sporidia. The fourth bar represents the period of germination of sporidia. The fifth bar represents the period of infection of pines. This diagram presupposes a large crop of recently matured telial columns.

Figure 3 starts with the same conditions as figure 1 but the cloudy weather is cut short by the appearance of the sun at the end of fourteen hours, which is presumably before any infection could take place. The fifth bar is omitted to indicate this fact.

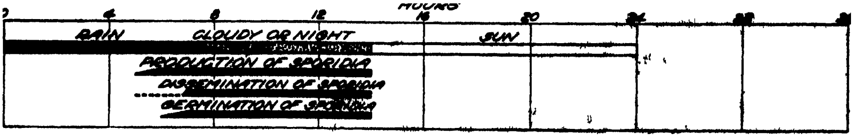

FIGURE 3. Bar diagram showing weather conditions which would presumably not result in the infection of *Pinus strobus* by the sporidia of *Cronartium ribicola*. The top bar represents the weather conditions. The second bar represents the period of production of sporidia. The third bar represents the period of dissemination of sporidia. The fourth bar represents the period of germination of sporidia. There is no fifth bar because no infection took place. This diagram presupposes a large crop of recently matured telial columns.

Figure 4 indicates a prolonged period of rain followed at once by sunshine. Production of sporidia reaches a maximum rate, but dissemination of the sporidia is almost entirely prevented by the falling rain which carries them down to the ground almost as soon as they are set free. A dotted line in place of a third bar indicates that a trace of dissemination may have taken place. Germination of the sporidia proceeds as long as they are moist, regardless of their location. Hence this bar is shown in full. Since the dissemination of the sporidia is reduced to a trace, the amount of infection which these few sporidia can cause is also reduced to a mere trace. The last bar consists of a dotted line to indicate this state of affairs.

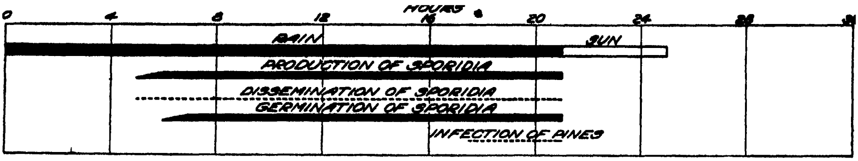

FIGURE 4. Bar diagram showing weather conditions which would presumably result in little or no infection of *Pinus strobus* by the sporidia of *Cronartium ribicola.* The top bar represents the weather conditions. The second bar represents the period of production of sporidia. The third bar is represented by a dotted line to indicate that the dissemination of sporidia is reduced to a mere trace. The fourth bar represents the germination of sporidia. The fifth bar is also represented by a dotted line to indicate that there was little or no infection. This diagram presupposes a large crop of recently matured telial columns.

Figure 5 indicates what happens when rain alternates with sunny weather, a thing which often occurs. Under these conditions sporidia may be formed and disseminated and begin to germinate, but the early appearance of the sun prevents their causing infection.

It should be stated that all five of these diagrams presuppose that there has recently been produced a large crop of mature telia which are ready to germinate under suitable conditions. As stated above, the time necessary for the telia to germinate increases with their age.

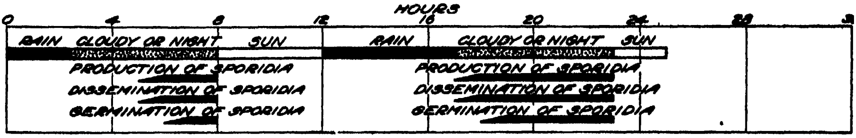

FIGURE 5. Bar diagram showing other weather conditions which presumably would not result in the infection of *Pinus strobus* by the sporidia of *Cronartium ribicola.* The top bar represents the weather conditions. The second bar represents the period of production of sporidia. The third bar represents the period of dissemination of sporidia. The fourth bar represents the period of germination of sporidia. There is no fifth bar because no infection took place. This diagram presupposes a large crop of recently matured telial columns.

It was intimated above that there are numerous questions in regard to the infection of pines by sporidia of *Cronartium ribicola* upon which we have little or no information. It may not be amiss to suggest some of these at this time. How long a time is required for all of the teliospores of a given generation to germinate? Are new telia formed during rain storms? Do immature telia mature during rain? How long a time is required for the formation of a mature telium? What period of time intervenes between the beginning and the crest of sporidial germination? Are sporidia which have already reached the needles of a pine washed off by rain? What is the minimum time for infection of pine needles after germinating sporidia are placed upon them? How long after the beginning of infection is the crest of infection? Why does not infection always occur when external conditions are apparently favorable to it? The number of such questions could be considerably increased, but these are sufficient to show some of the difficulties of the problem.

SUMMARY

Some of the factors influencing the period of production of the telia of *Cronatrium ribicola* are: weather conditions, time of season when the *Ribes* drop their leaves, and the varying ability of the species of *Ribes* to produce a second crop of leaves after the first has been dropped.

The germination of the teliospores is especially dependent upon moisture. Temperature *per se* does not seem to be so important; low temperatures merely inhibit or check the rate of germination. High temperatures have not been tested. Newly-matured teliospores germinate abundantly in about six hours at 75° F., while they require about twelve hours at 55–70° F. Increase in age increases the time needed for germination.

Longevity in this paper means the length of time during which teliospores remained ungerminated and still viable. Some of the factors influencing longevity of teliospores are: the habitat of the *Ribes* hosts, and the structure of the *Ribes* leaves. Both are closely associated with the access of moisture to the teliospores.

The factors which are necessary for the infection of white pines by *Cronartium ribicola* are many, and some of them are not known. It is known that there must be a period of sufficient moisture to germinate the teliospores, and that this must be followed by a period of high humidity during which the infection can take place. An attempt has been made to illustrate some of these conditions graphically.

Questions which need investigation are suggested.

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INOCULATION OF PINUS STROBUS TREES WITH SPORIDIA OF CRONARTIUM RIBICOLA

WALTER H. SNELL AND ANNIE RATHBUN-GRAVATT[1]

WITH TWO FIGURES IN THE TEXT

INTRODUCTION

From 1918 to 1922 the Office of Investigations in Forest Pathology carried out at North Conway, New Hampshire, a rather comprehensive program of field investigations of white pine blister rust. Part of the work was conducted by the present writers, under either the immediate direction or general supervision of Drs. Perley Spaulding and H. H. York. One phase of the work was the inoculation of *Pinus strobus* L. with sporidia of the white pine blister rust fungus, *Cronartium ribicola* Fischer. The present paper is a preliminary report of the successful inoculation of large trees in August and September, 1922.

Very little work has been done upon the conditions necessary for the infection of pines by sporidia, largely because of the difficulty of successfully inoculating them. The number of infections resulting from inoculations of white pines with *Cronartium ribicola* reported in literature is very small, and most of those reported have been on seedling pines. In 1912 Spaulding (6, 7) reported positive results from stem inoculations of young pines in the greenhouse. Clinton (1, 2, 3) was apparently the first American to secure infections through needles of a white pine as a result of artificial inoculation. Most of his successful infections occurred upon seedling pines in a greenhouse. Inoculations out-of-doors on trees 6 to 20 years old failed (2). In 1903, in Europe, Klebahn (5) successfully inoculated two young white pines under a bell jar. Tubeuf (8) also secured infection of young white pines. He found that he could infect 2-year old plants of *Pinus strobus* more readily than older ones.

1921 York and Snell (9) successfully inoculated 1918 and 1921 seedling pines out of doors in "iceless refrigerators" such as those described by Hunt (4). In less than three months after inoculation, some of the seedlings gave positive evidence of infection. Ultimately about two-thirds of them showed evidence of rust. In 1922 this experiment was successfully repeated by the writers.

Inoculations on large white pines at North Conway, however, had led to rather disappointing results. Some of the inoculations caused infection, but the percentage was too insignificant to give satisfactory data in the various studies. York's original method of inoculating large white pines was to germinate telial columns by floating them on the surface of water for about six hours, and then to enclose the telial columns and sporidia in leaf bundles of the pines. In 1922, however, the use of methods similar to those used in inoculating seedling pines (9) gave results which are more satisfactory.

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[1] Glenn G. Hahn assisted in part of this work in the summer of 1922.

METHODS AND CONDITIONS OF INOCULATION

The inoculating material used was collected from two patches of *Ribes nigrum* L. bushes, one in North Conway and the other north of Crawford Notch at Twin Mountain, New Hampshire, from some *R. odoratum* Wendl. bushes near Glen Station, New Hampshire, and from some *R. cynosbati* (L.) Mill. bushes at Jackson, New Hampshire. The method of inoculation used in 1922 simulated natural conditions as far as posssible. The greatest care was taken in selecting the inoculating material. The patches of Ribes were watched constantly for new generations of telia and, at the proper times, the leaves were picked and placed in an icebox, where they were kept for at least twelve hours before use. Usually at noon, or in special experiments, earlier or later, the infected leaves were cut in halves or quarters, according to size, and the pieces were placed in Petri dish moist chambers, such as previously described (9), for a period of six hours, to induce germination of the telia. At the end of this period, which was usually between 6 P.M. and nightfall, the dishes were carried to the previously selected field locations.

The places chosen for the inoculation of pines were in a region from which the *Ribes* had been eradicated in 1916. Thus the danger of natural infection of the experimental pines and that of the dissemination of the disease from the experimental plots to other pines were practically obviated. Within one experimental plot one white pine with a stem canker was found. This infection apparently occurred before the date of *Ribes* eradication.

The pines selected for inoculation were of two types: (1) small trees five to fifteen years of age, which could be entirely covered by moist chambers; and (2) the lower branches up to a point ten feet from the ground, of trees fifteen to over thirty years of age, which could be bent down so that the ends could be fastened under moist chambers. In the former case every twig of the tree, and in the latter every twig on the distal two or three feet of the branches, was inoculated.

Prior to inoculation, each branch was sprayed by means of a watering can (Fig. 1). Subsequently each twig was sprayed lightly with water by means of ar atomizer. The portions of *Ribes* leaves with the germinated telia were then applied to the clusters of needles which were pressed together

FIG. 1. The moist chambers used in inoculating large *Pinus strobus* trees with *Cronartium ribicola*. Note the size of trees used. Photograph by H. W. Snell.

loosely and fastened about half way round the clusters by small "invisible" wire hairpins (Fig. 2). This latter expedient proved of great convenience, because it not only provided a means for holding the leaf inoculum properly and securely in place, but also allowed ease and great rapidity in performing the work.

After inoculation the branches were again lightly sprayed and fastened in moist chambers. The moist chambers used were modifications of the "iceless refrigerator" described by Hunt (4). In all cases the moist chambers consisted of the same three parts—a reservoir, a support, and a cloth covering—which Hunt used. The reservoirs were dish pans or tubs of water. The covering consisted of two curtains—an inner one of thick, unbleached cotton cloth and an outer one of fine cheese cloth. York had found this combination most effective for keeping the curtains uniformly wet without wasting the water. Each curtain was tubular in form and was drawn together at the top with a puckering string. Various types of frames and forms similar to that described by Hunt have been used for supporting the reservoirs (Fig. 1), but these were found cumbersome to move about the woods. For this reason few of these frames were used in 1922. For most of the moist chambers the supports consisted of three stakes, cut on the spot in the woods, and driven into the ground until their tops were level. The reservoir was set upon the tops of these sticks, the curtains were hung in place, and sphagnum moss was placed upon the ground inside. Then the whole was wet and the inoculated branch enclosed within it. The use of stakes instead of frames made it much easier to use "iceless refrigerators" when making inoculations in the woods.

The inoculated branches remained in the moist chambers at least twenty-four hours, and sometimes thirty-six. Most of the experiments were begun at nightfall in order to take advantage of the naturally high humidity prevailing at night, because this prevented the evaporation of much water from the moist chambers. This arrangement made it possible not only to secure natural high humidity during the first stages of penetration of the needles by the sporidial germ tubes but also to obtain a second period of high night humidity, with only one intervening day of entirely artificially maintained humidity. At the end of twenty-four or thirty-six hours the

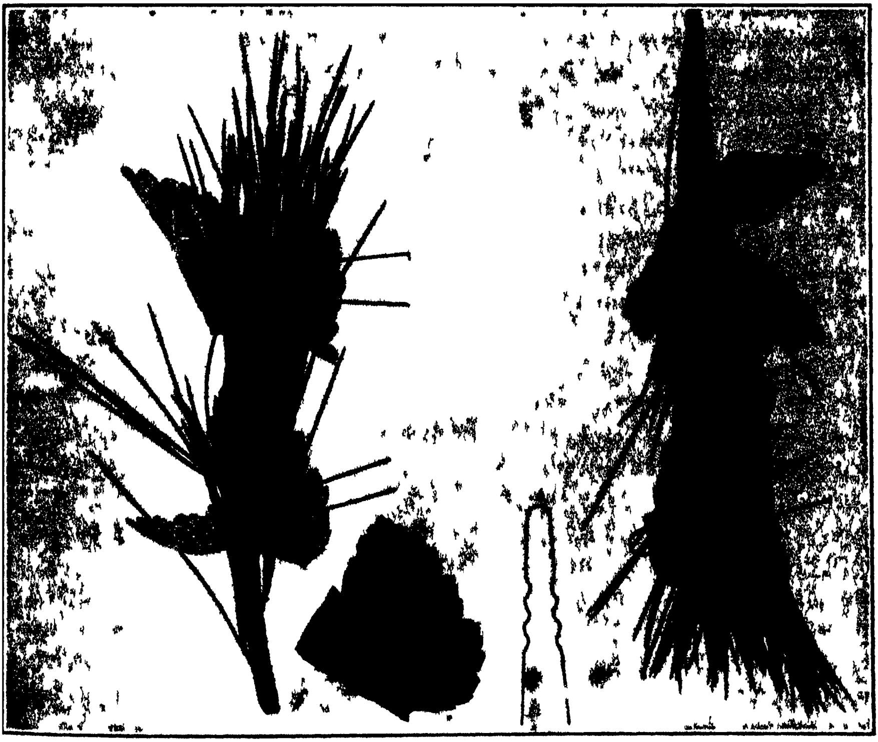

FIG. 2. Method of inoculating white pine twigs with *Cronartium ribicola.* The host from which the inoculum was secured was in this case *Ribes vulgare.* Note that the leaves were cut. Tho twig at right shows upper view; that at left shows lower one.

branches were removed from the moist chambers, and the inoculum was removed and buried.

The moist-chamber method of inoculation described above was used in inoculating 144 branches on 51 different white pines, ranging in age from 5 to more than 30 years. Most of them were over 15 years old.

Fifty-three additional branches on 9 of the same large trees were inoculated during the same period in the morning on different rainy days which promised to maintain a high atmospheric humidity. The method of inoculation was identical with that described above except that no moist chamber was used.

In addition, several small *Pinus resinosa* Ait. trees were inoculated during the same period to see whether or not the characteristic spots which indicate infection with *Cronartium ribicola* would form on their needles (2).

RESULTS OF THE INOCULATIONS

In June, 1923, the inoculated trees were inspected, and comparatively little evidence of infection was found. Characteristic needle spots (2) were observed on some of the inoculated branches. On one small tree nearly every needle showed characteristic spots. No twigs had become cankered. In September of the same year the senior writer again inspected them and found one definitely positive infection on a twig and several other doubtful ones. Some needle spots were also evident. In September, 1924, however, he found quite a number of positive infections on twigs and numerous indications that more positive ones might appear in 1925. The results of the inspection made in September 1924 are given in table 1.

Of the 51 trees inoculated in moist chambers, 18, or 35 per cent, showed cankers in 1924. Of the 144 branches inoculated, 20, or nearly 14 per cent, had developed cankers. On a few trees, only one twig of one branch was infected. However, most of the branches had cankers on from three to eleven twigs, and one branch had a canker on every twig inoculated. It is of interest to note here that the inoculations of trees over ten years old were twice as successful as those of trees between five and ten years of age.

Of the 9 trees inoculated on rainy days without moist chambers, 6 became infected in one or more branches. Of the 53 branches inoculated in this way, 11, or nearly 21 per cent, had become diseased. It is to be noted that a greater percentage of the inoculations not moist chambered than of those moist chambered were successful. The results of one season's inoculations do not prove that this difference is significant. On one tree six branches were infected, and on most of the infected branches there was more than one cankered twig.

No infections have yet been observed on needles or branches of *Pinus resinosa* inoculated with *Cronartium ribicola*.

TABLE 1.—*Results of inoculating native large white pines with the sporidia of Cronartium ribicola at North Conway, N. H., in 1922*

Age of inoculated trees (Yrs.)	Number of trees inoculated	Number of trees infected	Per cent of trees infected	Number of branches inoculated	Number of branches infected	Per cent of branches infected
			INOCULATED IN MOIST CHAMBERS			
5–10	7	2	29	50	4	8
10–over 30	44	16	36	94	16	17
Total	51	18	35	144	20	14
			INOCULATED WITHOUT MOIST CHAMBERS			
20–30	9[a]	6[a]	67	53	11	21
Total	51[a]	18[a]	35	197	31	16

[a] The inoculations which were not moist chambered were on the same trees with those which were moist chambered.

The data in regard to the source of the inoculum are not of great value because of the small number of tests with that from three of the sources. They are presented here for what they may be worth. In 16 tests with sporidia from *Ribes nigrum* collected at Twin Mountain, 18 per cent of the inoculations were successful. In 3 tests of those from *R. nigrum* collected at North Conway, 16 per cent were successful. In 3 tests with those from *R. odoratum,* 11 per cent were successful; and, in 2 tests with those from *R. cynosbati,* none of the inoculations were successful.

Inoculations were made upon both 1922 and 1921 needles, but the data upon their relative susceptibility are not yet complete. The evidence at present available is that the inoculations were as successful upon the two-year-old needles as upon the one-year-old needles.

The above results are presented because they represent the first really successful attempt to inoculate large white pines with the sporidia of *Cronartium ribicola.* It is realized that the percentage of successful inoculations is very small, but these results are much better than any attained before on older pines. They show that there is still a considerable gap in our knowledge either of the conditions of infection of *Pinus strobus* by *Cronartium ribicola,* of the periods of susceptibility of the pine needles, or of the viability of the teliospores or sporidia.

Later inspection may show considerably more infection than that found in 1924, as the period of incubation for *Cronartium ribicola* in the pine is sometimes several years.

SUMMARY

Account is given of the method used in inoculating large *Pinus strobus* trees with *Cronartium ribicola* at North Conway, N. H., in 1922.

Eighteen of the 51 trees inoculated in moist chambers had become diseased, and 20 of the 144 branches inoculated had become cankered in 1924.

Six of the nine trees inoculated under natural conditions, without subsequent moist chambering, had become diseased; and eleven of the 53 branches thus inoculated had become cankered in 1924.

The possibility of more infections appearing in 1925 or later is discussed.

No infection had occurred upon small *Pinus resinosa* trees in 1924.

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A PARTIAL EXPLANATION OF THE RELATIVE SUSCEPTIBILITY OF THE WHITE PINES TO THE WHITE PINE BLISTER RUST (CRONARTIUM RIBICOLA, FISCHER)

PERLEY SPAULDING

THE RELATIVE SUSCEPTIBILITY OF THE WHITE PINES TO THE BLISTER RUST

The writer has had an opportunity to see the white pine blister rust, *Cronartium ribicola* Fisher, in all of the principal outbreak areas of North America and in numerous localities of western and northwestern Europe. These extensive observations have led naturally to the making of comparisons of the behavior of this disease upon its different hosts and in different localities upon the same host. *Pinus cembra* L., the presumed original pine host of the blister rust, has two varieties which vary in susceptibility. The variety native in the Alps appears to be quite resistant; the variety native in Russia and Siberia appears to be quite susceptible. There is reason for believing that most of the *P. cembra* growing in western Europe is of the alpine variety; at any rate, it quite rarely becomes infected by blister rust. *P. cembra* is rather limited in distribution in Europe west of Russia, both naturally and as an ornamental. On the other hand, *P. strobus* L. is widely distributed over nearly all of this region, although it is thinly scattered as compared with a native species of similar, wide distribution, such as *P. sylvestris* L. General experience shows that *Pinus strobus* is much more commonly attacked by this disease in Europe than is any other species of pine. Indeed, it can be said that the distribution of the disease is practically identical with that of this tree. Because of their relatively small numbers and consequent limited distribution, *P. flexilis* James and *P. monticola* Douglas are not important hosts of the blister rust in Europe; the frequency with which the disease occurs upon the rather small numbers seen indicates clearly that they are both more susceptible than *P. strobus*.

American experience in British Columbia, where *Pinus monticola* is generally infected in its native habitat, shows plainly that this species is more susceptible than *P. strobus* growing in the same locality and out of its native range. This fully corroborates European experience, where both species are out of their native habitats. Very little *P. monticola* is known in the native range of *P. strobus*, but the few that have been seen are attacked by the blister rust. Comparison of the disease attacks upon these two species, when each is growing in its native habitat, also shows that *P. monticola* is more susceptible than is *P. strobus*. In this particular case, changed climatic conditions, within which both grow reasonably well, do not seem to influence their relative susceptibility.

As above stated, *Pinus flexilis* is more susceptible than *P. strobus* in Europe. Experience here in America is limited to a small comparative test (4) made by the writer. This indicated clearly that *P. flexilis* is more susceptible than is *P. strobus*. Under conditions foreign for both species, and under the conditions of the habitat of *P. strobus,* their relative susceptibility remains constant.

In another paper (7) the writer has ventured to make an estimate of the relative susceptibility of the white and pinon pines to the white pine blister rust. The disease is epidemic in North America only within the ranges of *Pinus strobus* and *P. monticola.* Hence American experience is extensive with these two species, but with no others. The disease is generally prevalent in the countries of western and northwestern Europe. It can be said then that all of the pines which are cultivated to any extent there have been subjected to infection by the disease. With this understanding of the situation one may comprehend the following grading of the pines.

TABLE 1.—*The estimated relative susceptibility of the white and pinon pines to the white pine blister rust.*

O. Immune, but too few tested for reliable estimate.

Pinus armandi Franchet.
" *bungeana* Zuccarini.
" *cembroides* Zuccarini.
" *monophylla* Torrey.
" *parryana* Engelmann.
" *edulis* Engelmann.

X. Resistant, but too few individuals, except of the first three, tested for reliable estimate.

Pinus cembra var. *helvetica*
" *excelsa* Wallich.
" *peuce* Grisebach.[1]
" *aristata* Engelmann.
" *balfouriana* Balfour.
" *koraiensis* Siebold and Zuccarini.
" *parviflora* Siebold and Zuccarini.

XX. Susceptible, but the last three species tested in limited numbers.

Pinus cembra var. *sibirica*[2]
" *strobus*
" *ayacahuite* Ehrenberg.
" *lambertiana* Douglas.
" *strobiformis* Engelmann.

[1] This estimate is based upon reports of resistance in Germany.

[2] This estimate is based in part upon reports of susceptibility in Russian literature.

XXX. Very susceptible, but first species tested only in limited numbers.
Pinus albicaulis Engelmann.
" *flexilis*
" *monticola*

As stated above, there are but a few species of the white and pinon pines which have been exposed to the attacks of the blister rust in large enough numbers to enable one to rank them in susceptibility with confidence. The species which have been tested in numbers are: *P. monticola, P. flexilis, P. strobus, P. lambertiana, P. excelsa, P. peuce, P. cembra* var. *helvetica,* and *P. cembra* var. *sibirica.* The ranking of the rest is purely tentative and liable to change as a result of future tests.

THE RELATIVE PERSISTENCE OF THE LEAVES AND DISTRIBUTION OF STOMATA ON THE LEAVES OF THE WHITE AND PINON PINES

The different species of white and pinon pines vary greatly in the length of time that they regularly retain their leaves. Data concerning this is taken from Sargent (5), Sudworth (8, 9), and Elwes and Henry (3).

TABLE 2.—*Persistence of the leaves of the white and pinon pines, and the distribution of the stomata upon the leaves.*

Species and susceptibility	No. of years the leaves persist	Stomata on the leaves
Pinus lambertiana XX	2–3	On inner and outer sides
" *peuce* X	2–3	" " side only
" *strobus* XX	2–3	" " " "
" *ayacahuite* XX	3	" " and outer sides
" *excelsa* X	3	" " " " "
" *parviflora* X	3	" " side only
" *bungeana* O	3–4	" " and outer sides
" *cembroides* O	3–4	" " " " "
" *monticola* XXX	3–4	" " " " "
" *parryana* O	3–4	" " " " "
" *cembra* X–XX	3–5	" " side only
" *edulis* O	3–5	" " and outer sides
" *koraiensis* X	3–5	" " " " "
" *strobiformis* XX	4	" " " " "
" *monophylla* O	4–5	20 lines of stomata
" *albicaulis* XXX	4–8	On inner and outer sides
" *flexilis* XXX	5–6	" " " " "
" *balfouriana* X	10–12	" " side only
" *aristata* X	12–14	" " " " "
" *armandi* O	(?)	" " " " "

It should be stated that, with most of the species having stomata on the outer surface of the leaves, the tendency is for the stomata on that surface to be rather few and to be located near the tip of the leaf.

CORRELATION OF SUSCEPTIBILITY WITH LEAF PERSISTENCE AND DISTRIBUTION OF THE STOMATA ON THE LEAVES OF THE WHITE AND PINON PINES

The writer's ranking of the pines in relative susceptibility was done in 1923, while preparing his notes upon the white pine blister rust in Europe for publication, and before any attention was given to the leaf persistence of the different species. For this reason it is believed that an unbiased opinion has been obtained, since the leaf persistence is based entirely upon published data of other investigators. In attempting to correlate susceptibility with leaf persistence, one is forced to eliminate some species because of too limited knowledge of their susceptibility, or include all with the distinct understanding that their grading is merely tentative and liable to change. The latter course seems better. The reliability of the estimates is indicated in table 1, so that there need be no misunderstanding concerning it. The available data may be presented in tabular form to the best advantage.

TABLE 3.—*The relative susceptibility, leaf persistence and distribution of the stomata upon the surfaces of the leaves of the white and pinon pines*

Pinus species	Susceptibility	Persistence of leaves in years	Distribution of the stomata on the leaves
albicaulis	Very susceptible	4–8	On inner and outer sides
flexilis	" "	5–6	" " " " "
monticola	" "	3–4	" " " " "
ayacahuite	Susceptible	3	" " " " "
cembra var. *sibirica*	"	3–5	" " side only
lambertiana	"	2–3	" " and outer sides
strobiformis	"	4	" " " " "
strobus	"	2–3	" " sides only
aristata	Resistant	12–14	" " " " "
balfouriana	"	10–12	" " " " "
cembra var. *helvetica*	"	3–5	" " " " "
excelsa	"	3	" " and outer sides
koriaensis	"	3–5	" " " " "
parviflora	"	3	" " sides only
peuce	"	2–3	" " " " "
armandi	Immune	?	" " " " "
bungeana	"	3–4	" " and outer sides
cembroides	"	3–4	" " " " "
edulis	"	3–5	" " " " "
monophylla	"	4–5	20 lines of stomata
parryana	"	3–4	On inner and outer sides

It has been shown that most of the infection of white pines by the white pine blister rust starts in the leaves, and that entry to the leaf is through the stomata (1). It has also been determined that the fungus requires several months to reach the bark of the twig after infection is established within the leaf. Tests (1, 6, 10) show that with *Pinus strobus* the second year leaves may take infection about as readily as the first year ones. One may say that a considerable percentage of all of the infections take through the older leaves on both *P. strobus* and *P. monticola*. With *P. strobus,* there is a chance that the older infected leaves may drop before the fungus reaches the bark of the twig. This is believed to happen in many instances. Since *P. monticola* retains its leaves a year longer than *P. strobus* does, a much larger number of the infections upon the older leaves will succeed in reaching the twig. Considering only the leaf casting, it is quite certain that those species of pines which retain their leaves for longer periods will prove to be more susceptible. Turning to table 3, what are the facts? In the first group of three "very susceptible" species, the average leaf persistence is decidedly longer than is that of the second group of five "susceptible" species, the ratio being about 5 to 3 years. This difference in years of persistence is believed to be significant. It can not be said that the two last groups show any relation between susceptibility and leaf persistence. Evidently some other factor is exerting a greater influence.

The writer would call attention especially to the characters of *P. strobus* and *P. monticola,* because of the great similarity of the two species. They are very similar in the relative size and shape of the crown, in the size, number, and distribution of the branches and twigs, and in the whorled arrangement of the branches upon the trunk. The latter, however, gives an impression of greater density and amount of foliage. This seems to be due to the twigs being clothed with at least one year's more leaves. Both of these species have been subjected to infection in their native habitats, so that their relative susceptibility is well established.

An examination of the possible influence of the stomata upon susceptibility results in the finding of the following facts. There is no data upon the number of stomata upon the leaves of the different species of pines; except statements that some of the species have a given number of rows of stomata on the inner surfaces of the leaves. The number in a row may, and probably does, vary much. There are certain species which have stomata only upon the inner surfaces of the leaves, while there are some which also have them upon the outer surface. The stomata upon the outer surface of the leaves are usually located toward the tip of the leaf. Since there is no definite indication of the actual number of stomata for the different species, it seems reasonable to assume that those species which have stomata upon the outer surface of the leaves also have larger numbers of stomata. It is apparent that a leaf which has stomata upon all surfaces is more apt to receive sporidia in close enough proximity to one so that infection will succeed, than is a leaf which has one-third of its surface without such openings for infection. This difference appears to be one which may be significant and is the basis of the present correlation. Turning to table 3, we find that all of the "very susceptible" species have stomata upon all of the leaf surfaces. The "susceptible" group of five species has three species with stomata upon all surfaces of the leaves, and two with them upon the inner surfaces only. The "resistant" group has two out of seven species with stomata upon all surfaces of the leaves. The "immune" group includes the pinon pines, which are not known to become infected by this fungus. If these be excluded, there are left two species, one of which has stomata upon all surfaces of the leaves, while the other has them upon the inner surfaces only. In the first three groups of table 3 there appears to be a correlation between the distribution of the stomata on the leaves and susceptibility of the species. This is more apparent when one remembers that the rated susceptibility of more than half of the species in the "resistant" and "susceptible" groups is liable to be raised by future tests.

THICKNESS OF INNER BARK A POSSIBLE FACTOR IN SEVERITY OF ATTACKS OF THE BLISTER RUST

Pinus strobus, in exceptionally severe attacks of the blister rust, may have many twigs killed by the fungus. Trees killed by it die from the girdling of the stem, however, not because all of the twigs are killed. *Pinus monticola* often dies from the effect of having all of the twigs killed. There appears to be a real difference in intensity of attack in the small twigs. On branches and stems there is a similar difference; on *P. strobus* the pycnia are usually separate and distinct from one another; on *P. monticola* they coalesce into continuous blotches or sheets. The amount of liquid exuded is decidedly greater with the latter also. There is thus good evidence that the fungus vegetates in the bark of *P. monticola* as it never does in the bark of *P. strobus.* There is a decided difference in the thickness of the smooth bark of the two species, that of *P. monticola* being thicker. The fungus vegetates largely in the inner bark tissues (2). Bark which has large amounts of such tissues would appear to favor such a fungus. The blister rust fungus certainly flourishes in trees which are especially vigorous in growth (1). The writer believes that the greater abundance of phloem in the bark of *P. monticola* directly favors the growth of the fungus.

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THE LOOSE SMUT OF RYE (USTILAGO TRITICI)

HARRY B. HUMPHREY and VICTOR F. TAPKE

WITH THREE FIGURES IN THE TEXT

INTRODUCTION

Rye in North America is subject to several disease-producing fungi, three of which are smuts. Of these, stem smut, *Urocystis occulta* (Wallr.) Rab., is of most frequent occurrence and greatest economic importance in the United States. The other smuts are loose smut, *Ustilago tritici* (Pers.) Jens., and bunt, *Tilletia tritici* (Bjerk.) Wint., the latter of which, according to Gaines and Stevenson (4), was observed by them at Pullman, Wash., on rye and on some wheat-rye hybrids. *Tilletia secalis* Kuehn may be synonymous with *T. tritici* though we are without the necessary cultural evidence to prove their identity. It is the opinion of Gaines and Stevenson (4) that they are one and the same species.

Although Kaspar Bauhin (1), in listing what he conceived to be species of *Ustilago,* included *U. secalena,* he does not describe it. We therefore are without any information as to which smut or other fungus he refers. In 1849, Rabenhorst (6) described as *Uredo secales* an odorless smut which, according to his description, is not an *Ustilago.* The *Ustilago secalis* Rabh. described by Fischer von Waldheim (2) apparently is synonymous with Rabenhorst's *Uredo secales.* It therefore seems quite probable that the occurrence of loose smut in rye was first recorded in 1914 (5) following its discovery in a field of spring rye on the Dickinson (N. Dak.) Substation in July, 1913. Vavilov (7) records his observations of this smut on rye in Persia, in 1916, and refers it to *Ustilago tritici* (Pers.) Jens. The records of the Office of Pathological Collections and Plant Disease Survey show a wide distribution of loose smut on rye in the United States. Since its discovery in North Dakota, it has been reported from the following states: Illinois, Indiana, Kentucky, Minnesota, Missouri, New York, Oklahoma, Virginia, West Virginia, and Tennessee. The frequency of its occurrence, however, has not been such as to give the disease more than a scientific interest. Howver, there always is the possibility of its becoming of economic importance through the introduction of more highly susceptible varieties of rye or through the development of virulent physiologic forms of the organism.

THE DISEASE

Symptoms.—Previous to heading there generally is no evidence by which a rye plant infected by loose smut may be recognized. Occasionally, however, streaks of smut may develop in the upper leaf before the head emerges,

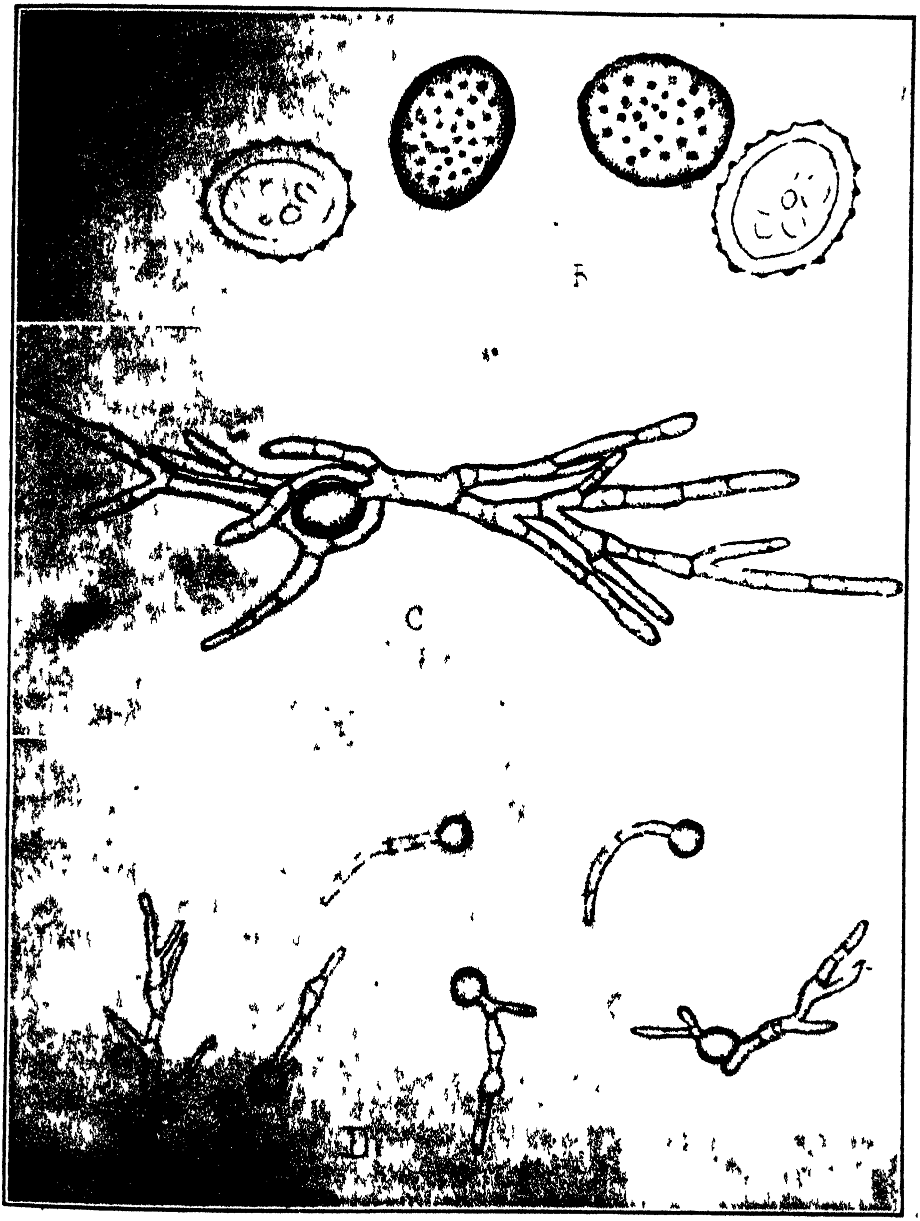

FIG. 1. A. Spores of *Ustilago tritici* from a smutted head of wheat. ×2400. B. Spores of *U. tritici* from a smutted head of rye. ×2400. C. An individual plant of *U. tritici* from a single spore taken from a smutted rye head. ×1000. D. Sporelings of *U. tritici* from a culture made from a transfer of spores from a smutted head of rye. ×500.

especially when the plants are grown in the greenhouse. Except as noted above, no parts of the plant other than the spikelets are affected. When the diseased head has emerged from the boot, the spikelets are seen to be almost completely destroyed and replaced by an odorless, powdery dark-brown mass of smut spores. In cases of partial smutting of the head, as noted below, some of the spikelets at the juncture of healthy and diseased tissues may be only partly destroyed. The effects of *Ustilago tritici* on heads of rye, therefore, are similar to those on heads of wheat, except that in rye partial smutting of the head seems to be the rule (Fig. 2), whereas in wheat (Fig. 3) it is the exception. In rye, as in wheat, partial smutting of the head always proceeds from the base upward, and in such cases all stages from complete to partial destruction of the floral parts will be found progressively from the base to the tip of the head (Fig. 2). Although the lower spikelets on a head of rye may be partially or wholly destroyed, the behavior of the flowers in the upper spikelets and the production and maturation of their seed proceed apparently in the normal manner.

INVESTIGATIONS

Methods.—A careful study of the behavior of the rye-smut fungus in artificial culture and a comparison of its morphologic characters with those of authentic *Ustilago tritici* led the writers to suspect the identity of the two smuts (Fig. 1). The spore characters of the two smuts were optically identical. An extensive series of spore measurements of the rye smut were made and compared with those of spores obtained from a smutted head of wheat. In both cases, when observed under high magnification, the spores were a pale olive-green color, somewhat lighter on one side, slightly oval, measuring from 5 to 9 µ in the greater diameter, and minutely but sparsely echinulate. Germination of the rye-smut spore, as in the case of the wheat-smut, proceeds with the development of a germ tube, often curved, which in water or nutrient solutions may become 5–7 septate and produce several branches. In no instance was there observed the development of any sporidia in cultures of either smut.

But, in order to establish beyond doubt the fact of their identity, some cross-inoculation experiments in the greenhouse and field were made in 1922 and repeated in 1923. These experiments involved the transfer of loose-smut spores from smutted heads of wheat to the ovaries of normal rye plants. Reciprocal cross inoculations also were made. Following is the method of inoculation employed. Heads were selected on plants of Fultz (C. I. 1923), Leap (C. I. 4823), and [illegible] (C. I. 2980) wheat grown singly in pots in the greenhouse. Only those heads were chosen which contained a relatively large number of flowers in the stages of bloom indicated

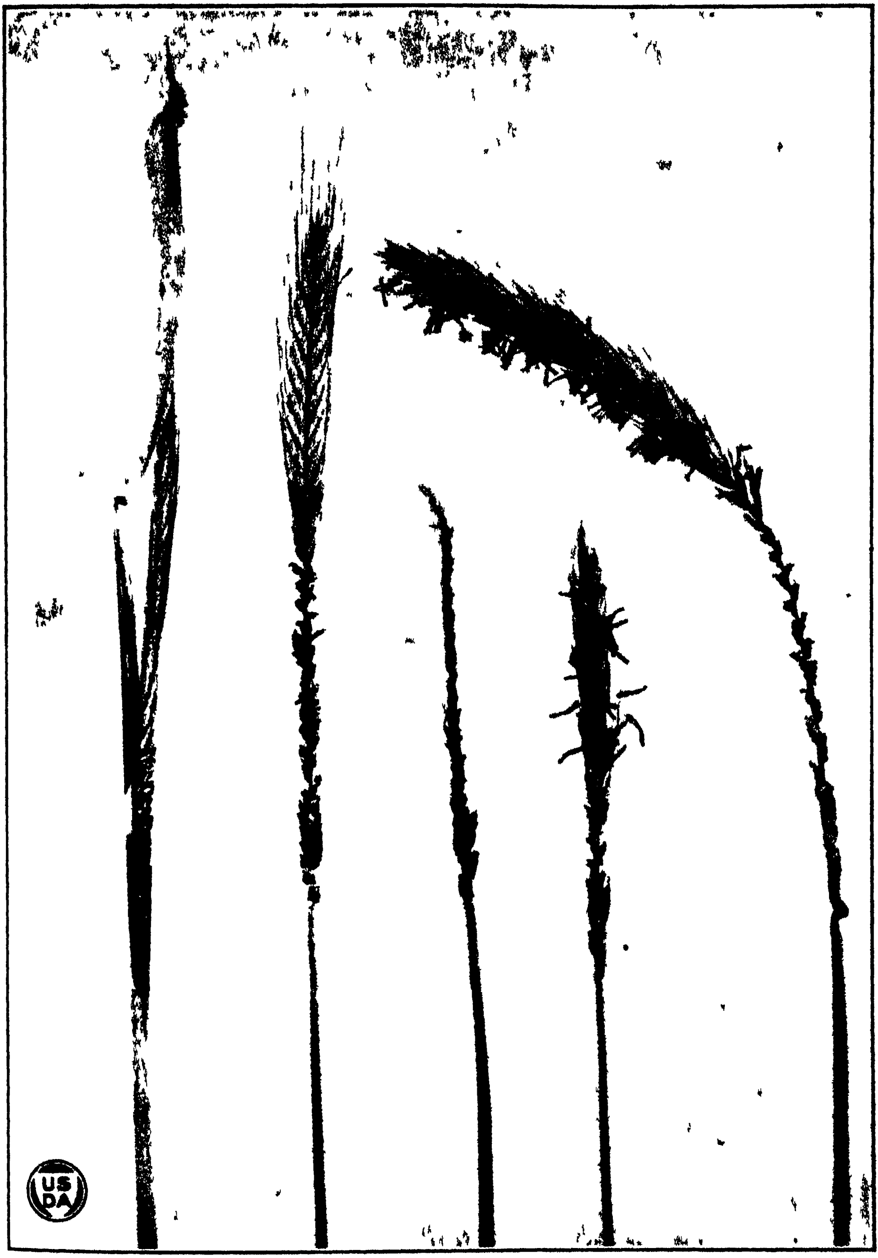

FIG. 2. Heads of rye infected with loose smut. The characteristic partial destruction of the head is here clearly shown. The seed from which these heads were grown was infected with *U. tritici* from wheat.

below. The flowers were gently opened with forceps, the tips of which had been dipped in a mass of smut spores from one or more heads of Rosen rye (C. I. 195), collected in the field on the day the inoculations were made. If the pollen of the flower was (1) about to be shed, as indicated by its color at maturity in the particular variety at hand, or (2) if the pollen was being shed, or (3) but recently shed, as indicated by a freshly plumose stigma and an ovary not noticeably enlarged, a small amount of inoculum was applied by lightly touching the stigma with the spore-laden tips of the forceps. All flowers not in the condition indicated in the foregoing were removed. The period of floral development within which the inoculations were made, therefore, fell within the limits determined by Freeman and Johnson (3) during which infection may take place in wheat flowers inoculated with spores of *Ustilago tritici*.

The inoculations were made at a time when no heads of wheat affected with loose smut were present in the near-by fields or greenhouses. However, in order to determine positively whether or not loose-smut infection might, by chance, have taken place naturally from spores blown into the greenhouse from without, several uninoculated heads were left to serve as controls on each of the plants which supplied heads for inoculation. After inoculation the plants were removed at once to an adjacent room, kept separately enclosed therein for one week, and then returned to the greenhouse. To serve as a control, a number of plants of Fultz (C. I. 1923) and Stoner (C. I. 2980) were inoculated in the greenhouse later on the same day, with loose-smut spores from freshly collected heads of Goens wheat, and the variety Leap (C. I. 4823) was similarly inoculated four days later. The technique here employed was precisely the same as that used in the above inoculations with loose-smut spores from rye. Following the inoculations of these plants, they too were removed to an adjacent room, kept separately enclosed therein for one week, and then returned to the greenhouse.

Methods were used in the inoculation of rye in 1922 similar to those described for wheat. Heads containing a relatively large number of flowers in the same stage of bloom as was described above were selected on plants grown in a plat at Arlington Experiment Farm, Rosslyn, Virginia. In one section of the plat, heads were inoculated with loose-smut spores from Rosen rye (C. I. 195) and, in another section of the plat, with loose-smut spores from Goens wheat. The diseased heads of wheat and rye used for sources of inoculum were collected in the field the same day the inoculations were made. When the inoculated rye heads were collected at maturity, a number of neighboring uninoculated heads also were collected in order to determine whether or not any loose-smut infection might have taken place naturally by means of spores blown from distant fields of wheat or rye.

Results.—Table 1 shows the following results: (1) High and similar percentages of infected plants and heads were obtained in wheat inoculated with loose-smut spores from wheat and from rye. (2) In rye, the percentages of infection also were about the same regardless of the source of inoculum. In all cases, the percentages of smutted heads of rye were less than 1 per cent when the plants were grown in the field. When grown under greenhouse conditions, however, the infection was more abundant.

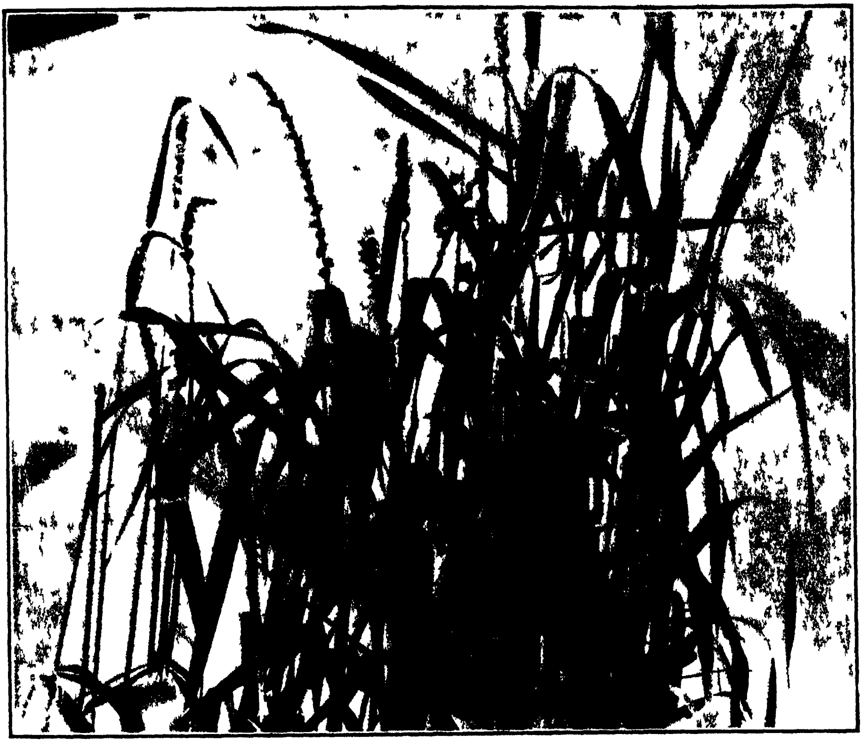

FIG 3 A greenhouse culture of wheat grown from seed infected with *U tritici* from rye.

The inoculation of rye with loose-smut spores from wheat was repeated in 1923 with some difference in method. Three or four heads, each on a near-by plant and each in approximately the same stage of development, were enclosed in a paper bag before anthesis of the flowers. The bagged plants were in plats of Rosen (C. I. 195) and Rimpau rye (C. I. 126). During the period of anthesis, a head was removed from each bag, inoculated, and immediately replaced. The inoculum consisted of loose-smut spores from Goens wheat, collected in the field on the day of inoculation.

TABLE 1.—*Number of plants and heads and number and percentage es of smutted plants and heads of three varieties of wheat and two varieties of rye inoculated in the flowering stage with spores of loose smut from wheat or from rye, in 1922 and 1923, at Arlington Experiment Farm, Va.*

Crop and variety	C. I. No.	Source of inoculum	Date of inocu-lation	Inocula-tion in field or greenhouse	Crop, field or green-house	Number of plants Total	Number of plants Smutted	Percent-age of smutted plants	Number of heads Total	Number of heads Smutted	Percent-age of smutted heads
			1922								
Stoner wheat	2980	Bosen rye	May 18	Greenhouse	Greenhouse	43	28	65.12	94	46	48.94
do	do	Goens wheat	do	do	do	96	52	54.17	230	87	37.83
do	do	Not inoculated	....	....	do	60	0	0.00	....	....	....
Fultz wheat	1923	Bosen rye	May 18	Greenhouse	do	78	43	55.13	218	105	48.17
do	do	Goens wheat	do	do	do	91	53	58.24	221	122	55.20
do	do	Not inoculated	....	....	do	57	0	0.00	....	....	....
Leap wheat	4823	Bosen rye	May 18	Greenhouse	do	11	2	18.18	35	4	11.43
do	do	Goens wheat	May 22	do	do	86	8	9.30	274	10	3.65
do	do	Not inoculated	....	....	do	60	0	0.00	....	....	....
Bosen rye	[illegible]	Bosen rye	May 18	Field	Greenhouse	38	8	21.05	162	12	7.41
do	do	Goens wheat	May 16	do	do	11	2	18.18	72	2	2.78
do	do	Not inoculated	....	....	....	187	0	0.00	....	....	....
do	do	Bosen rye	May 18	Field	Field	68	2	2.94	792	2	0.25
do	do	Goens wheat	May 16	do	do	15	1	6.67	171	1	0.58
do	do	Not inoculated	....	....	do	187	0	0.00	....	....	....
			1923								
do	do	Goens wheat	May 22	Field	Greenhouse	22	0	0.00	103	0	0.00
Rimpau rye	126	do	do	do	do	157	3	1.91	475	4	0.84

The inoculated and uninoculated heads of wheat and rye were collected at maturity and thrashed by hand. In the greenhouse, the seed was sown at intervals of 2 inches in rows 6 inches apart on October 28, 1922, and November 13, 1923. In the field, the seed was sown at intervals of 7 inches in rows 1 foot apart on October 23, 1922. The results are presented in table 1.

VARIETAL SUSCEPTIBILITY

Reports on the occurrence of loose smut in rye have afforded no information as to the variety on which the disease was found.

In 1922, 1923, and 1924, the writers inspected all of the fortieth-acre agronomic plats of rye of the Office of Cereal Investigations at Arlington Experiment Farm. Each year a few heads of loose smut were found but only in the varieties Rosen (C. I. 195) and Rimpau (C. I. 126). Varieties in which no loose smut was found are the following: Abruzzes (C. I. 40), Abruzzes (C. I. 40–1), Giant Winter (C. I. 30), Henry (C. I. 138), Ivanoff (C. I. 34), Mexican (C. I. 108), St. Johns (C. I. 130), Virginia (C. I. 128–1), von Ruemker (C. I. 133), von Ruemker (C. I. 173), and Winter (C. I. 208).

SUMMARY

The occurrence of loose smut in rye was discovered in North Dakota in 1913 and recorded in 1914. Since then loose smut has been observed in Illinois, Indiana, Kentucky, Minnesota, Missouri, New York, Oklahoma, Tennessee, Virginia, and West Virginia.

Comparative cultural and microscopic studies of this smut and the loose smut of wheat failed to show any difference between them.

The reaction of the rye plant to invasion by *Ustilago tritici* is similar to that of wheat except that, in rye, total destruction of a part (often the lower third or half) of the head is the rule, while in wheat complete destruction of all florets is the rule.

Cross-inoculation experiments in which heads of both wheat and rye were inoculated with spores of loose smut from rye and from wheat, respectively, aided in establishing the identity of the two smuts.

Varietal-resistance observations indicate that of 13 varieties and selections only two, Rosen (C. I. 195) and Rimpau (C. I. 126), are susceptible.

OFFICE OF CEREAL INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE

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A STORAGE ROT OF PEACHES CAUSED BY A NEW SPECIES OF CHOANEPHORA[1]

E. D. EDDY

WITH ONE FIGURE IN THE TEXT

The recent investigations on the causal organisms of decays of fruits or vegetables during transit, storage, or at the markets, have brought the Mucorales to the attention of phytopathologists. Forms which were supposed to be mere saprophytes and worthy of little consideration by investigators of plant diseases are rapidly being added to the list of fungi capable of producing rots. With the exception of *Rhizopus nigricans* and *Choanephora cucurbitarum* (B. & Br.) Thaxter, species which have long been recognized as of importance as facultative parasites, the Mucors have been disregarded by the majority of writers on this subject.

Rhizopus nigricans is the most frequently found and most widely distributed species of this group. Its importance as the cause of plant diseases is partly due to great numbers of its spores which may be found anywhere in the air. Many other species of the Mucors, belonging to various genera, may produce decays of plant tissues under suitable environmental conditions. But they can never attain the same importance as the common "bread mold," as their spores are rarely present when such conditions occur in nature.

Storage rots have been attributed to a number of species of the genus *Mucor*. Harter in 1918 found that *Mucor racemosus* was the cause of a slow decay of sweet potatoes during storage at low temperature. *Choanephora cucurbitarum* is the causal organism of a soft rot of young squashes on the vines as well as of the mature fruit during storage. At the New York Markets Laboratory of the U. S. Department of Agriculture, isolations were made from the decayed fruits or vegetables which were found at the New York markets. Although inoculation experiments were not carried out in every case to prove the relation between the organism isolated and the rots, these isolations would indicate that a number of species should be considered as facultative parasites. At least six different species of *Rhizopus* were found causing decay. Four species of *Mucor* were obtained many times. *Choanephora cucurbitarum* was often isolated from squashes. And *Syncephalastrum racemosum* was twice isolated from decayed oranges.

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[1] The work discussed in this paper was done in 1919 as a cooperative enterprise between the Office of Cotton, Truck, and Forage Crop Disease Investigations and the Bureau of Markets, United States Department of Agriculture, through the courtesy of the Director, Dr. C. S. Gager, in the laboratories of the Brooklyn Botanic Garden, Brooklyn, N. Y.

This paper deals with a new species of this group of fungi. It is closely related to *Choanephora cucurbitarum*. Although it may never become of serious importance on account of its limited distribution, it is of interest as another species of the Mucors which may develop as a facultative parasite and also because of its position in the systematic arrangement of these fungi.

The organism was isolated from decaying peaches found on the New York market by Dr. Mix during the summer of 1918. Pure cultures from a single spore were obtained and kept growing until the fall of 1919 before inoculations were made to determine whether it was the casual organism of this decay. Although this form has not been previously described, it is very similar or perhaps identical with a form isolated by Blakeslee and referred to by Thaxter[2] in his discussion of the various species of *Choanephora.*

The fungus grows well on various artificial media. A very slight white mycelial growth is noticeable covering the surface of the medium only during the first day or so of growth before the mycelium becomes black due to the formation of many small sporangia. The fertile hyphae arise directly from the surface of the substratum, without branching in any way, to produce a single spherical, black sporangium at the apex (Fig. 1C). This mode of growth largely determines its appearance in culture. On potato or malt-agar the growth is black and rarely more than $\frac{1}{8}$ inch high. It develops more luxuriantly on bread, reaching a height of from $\frac{1}{4}$ to $\frac{1}{2}$ inch. The fungus has been cultivated for eighteen months and still fruits just as abundantly in culture as it did when first isolated. It does not appear to lose its power of spore production when grown on artificial media as is the case with the other species of *Choanephora* that have been described. Chlamydospores of the fungus are illustrated in figure 1B.

The mycelium from these pure cultures was used for inoculating peaches to determine whether this species would produce a soft rot similar to that from which it was first isolated. The surfaces of seven healthy peaches were sterilized by dipping each fruit in alcohol to remove any air and then letting them stand for about five minutes in mercuric chloride solution (1:-1000). After this treatment they were rinsed thoroughly in sterilized water. Incisions through the skin were made with a flamed scalpel and bits of mycelium were inserted in each incision on five of the fruits. The two other peaches were not inoculated and served as controls. All the specimens were then placed in sterilized glass jars and kept at room temperature.

The five inoculated peaches were entirely decayed at the end of seven days and the fungus was fruiting abundantly over the surface, while the two control specimens had remained in perfect condition. The rot first appeared on the second day as a small discolored and softened area on the surface immediately surrounding the point of infection. A soft brown decay spread rapidly, involving the entire fruit in from four to six days after its first appearance. The sporangia developed on the surface forming a low black felt about an eighth of an inch thick. There was no white mycelial growth, "whiskers," over the surface as is commonly the case with *Rhizopus nigricans* or *R. reflexus* when growing under moist conditions. Although softened, the tissues remain fairly firm and do not collapse and "leak," as when decayed by species of *Rhizopus*.

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[2] Thaxter, R., New or peculiar Zygomycetes, 3: *Blakeslea, Dissophora,* and *Haplosporangium,* nova genera. Bot. Gaz. 58: 353–366. 1914.

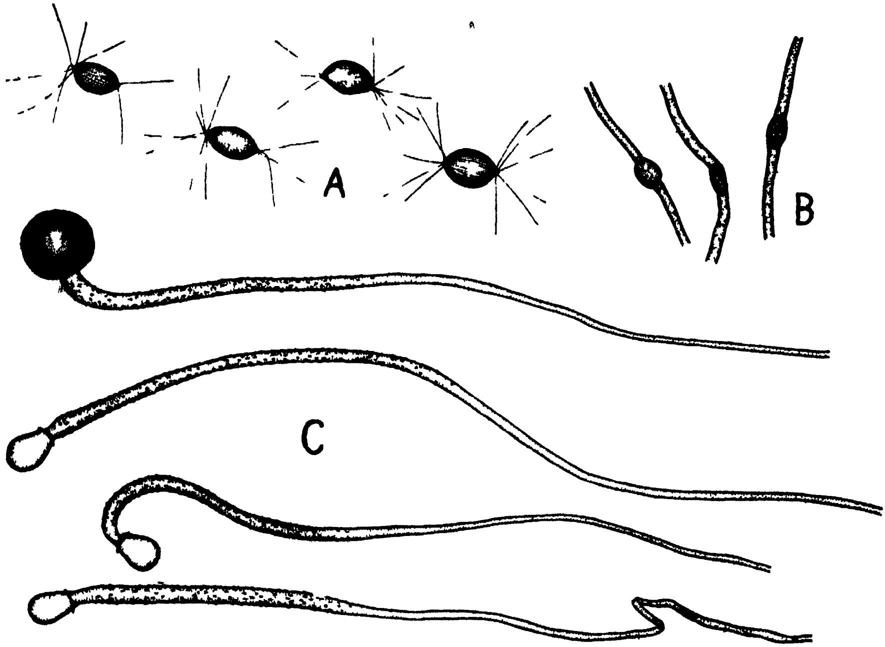

FIG. 1. *Choanephora persicaria.* A, Four spores, magnified 870 diameters; B, Chlamydospores, magnified 385 diameters; C, Four fertile hyphae showing sporangia and columellae of *Choanephora persicaria,* magnified about 49 diameters.

Choanephora persicaria was thus shown to be a facultative parasite, capable of producing a soft rot of peaches under experimental conditions. Infection may occur through a bruise or cut when the environmental conditions are such that the injured surface remains moist and penetrable long enough for the spores to germinate and the hyphae to enter the tissues. F riments were not tried to determine whether this fungus might penetrate an uninjured surface. The results would probably be negative as with the other forms of the Mucorales which act as facultative parasites.

Choanephora persicaria n. sp.

Mycelial growth low, black, 2.5–12 mm. high. Fertile hyphae rising directly from the mycelium, erect, unbranched, 0.5–2 mm. high, terminated by a single sporangium, outer surface conspicuously roughened in upper half of its length and distinctly swollen. Sporangia spherical, erect, nodding or circinate, variable, 64–128 μ in diameter, average 100 μ. Sporangial membrane covered with fine crystals, dehiscent and not quickly diffluent, having a narrow collar at the base of the columella. Columella elongated, constricted at the base, pyriform to globose, hyaline, 76–121.5 μ long × 49–83.6 μ broad, very variable in shape and size. Spores oval or elliptical, elongated, often irregular in shape, indistinctly longitudinally striated, hyaline or very slightly darkened, 19–22 × 11–15 μ, bearing a few exceedingly fine, radiating appendages in groups at either pole. Length of appendages equal to that of the spores or greater. "Conidia" or sporangiola not observed. Chlamydospores cylindrical, hyaline, 15–26.6 × 11–16 μ, very variable in shape and size, and not numerous.

Isolated from decayed peaches found on the New York market.

The affinities of *Choanephora persicaria* are certainly with the species of *Choanephora* that have so far been described and with *Blakeslea trispora* Thaxter. The spores of both of these genera show the fine appendages which radiate from either pole and also the strictions on the surface (Fig. 1A). The spores of the other species are colored, purple or reddish-brown. The circinate normal sporangia, which are produced in great numbers in this form, very closely resemble the normal sporangia produced occasionally by the other species. But in this species only one type of sporangia has been found, while the "conidia," the stage usually developed in other species of *Choanephora,* or the sporagiola as found in *Blakeslea,* have not appeared.

Thaxter refers to a strain showing this same characteristic and writes: "At least one species of Choanephora is known to the writer which was isolated by Blakeslee during his investigations on the Mucorales, and has never been seen to produce anything but normal sporangia with the typical appendiculate spores of this genus, although it was kept in cultivation for a period of years."

A number of attempts were made to induce the development of another spore-form, "conidia" or sporangiola, by growing the organism in open dishes in order to lessen the degree of humidity, as in related forms the "conidial" stage develops best, or only under dry conditions or in a free circulation of air. None but "normal sporangia" have been found in any cultures. The apparent absence of any "conidial" stage in this species is not sufficient basis for a separation from the species which it so closely resembles in all other characteristics.

The writer wishes to acknowledge his indebtedness to Dr. Mix, who isolated this fungus, and to Dr. Roland Thaxter for valuable suggestions.

PRELIMINARY STUDIES ON THE CONTROL OF CEREAL RUSTS BY DUSTING

C. V. KIGHTLINGER

During the season of 1924 the writer undertook some experimental work on the use of fungicidal dusts in the control of cereal rusts. The results of these preliminary studies are so striking and promising that it seems desirable to present a brief summary for the benefit of other workers interested in the problem of cereal rust control.

While in its broader aspects the problem might well have involved control by the application of fungicidal protectants in any form, spraying was excluded because of its evident impracticability. The use of a fungicidal dust seemed most likely to yield results of practical value.

Knowing that sulfur had proved effective as a protectant against rust infection in two well established cases (the rusts of asparagus and of snapdragons), it was believed that most probably sulfur would prove effective as a protectant against rust in the case of cereals. After numerous laboratory tests on the inhibitory effects of several sulfur dusts on the germination of uredospores of *Puccinia coronata* it was decided to use dusting sulfur. The dust used was 90–10 sulfur lead arsenate dust, because it was the only kind on hand in quantity when dust was needed. However, it is not assumed that the lead arsenate contained in the dust had any marked fungicidal value.

The aim of the work was to determine whether or not sulfur would prevent rust infection of cereals. This part of the work fell naturally into three phases: (1) laboratory studies on spore germination in relation to the fungicidal efficiency of sulfur dusts, (2) dusting tests in the greenhouse on growing plants, (3) tests of the efficiency of dusts on plants under field conditions. The results obtained from the laboratory tests on germination of uredospores in drop cultures on dusted and undusted slides afforded data for the study of the comparative values of the several dusts with respect to their inhibitory effects. It also provided a criterion for the elimination of the less desirable forms in future work. An average of 72.9 per cent germination of uredospores of *P. coronata* was obtained in checks as against 18.8 per cent on the slides dusted with the 90–10 mixture. However, these results were not held to be absolutely the same as those which might be obtained in the field, although past experience with this method, originally developed by Wallace and Reddick, has shown it to be very reliable in this respect.

Among other things of interest observed in the germination tests was the fact that the germ-tubes produced on slides dusted with sulfur were shorter than those in the checks. Those on slides dusted with 90–10 dust, averaged 35 µ in length and those with precipitated sulfurs 58.123 µ, while in the case of the check the average length was 116.5 µ. It was further noted that the germ-tubes produced on dusted slides were very much distorted and vacuolate, and very unevenly granular, indicating, it seemed, that the fungicide while not entirely preventing germination was decidedly injurious to those germ-tubes which did form. It appeared probable that such germ-tubes could never produce infection. This was later confirmed by the greenhouse and field tests. Spores nearest the edge of the drops where they came in closest contact with the sulfur particles seldom, if ever, germinated.

In the greenhouse work 90–10 dust only was used. The larger part of the work in the greenhouse was conducted with *P. coronata* on oats though tests with *P. graminis avenae* gave equally good results. The oats for these tests were grown in pots. They were dusted with the same duster with which the slides in the laboratory had been dusted so that the applications on slides and leaves were approximately equal as to amounts per unit area. After the plants had been dusted, the spore suspension was applied with an atomizer, following which the plants were placed in a moist chamber for as long a time as appeared necessary, depending upon the conditions obtaining at the time of the treatment.

With these pot cultures striking results were obtained. Out of a total of 2,071 inoculated plants 1,057 were dusted, and 1,014 not dusted. The 1,057 dusted plants showed but two lesions or sori, while the 1,014 non-dusted ones showed 4,517 sori.

The results obtained from these greenhouse tests were extremely suggestive and stimulating. In order to determine whether the results obtained in the greenhouse could be duplicated or approximated under field conditions, experiments were made during the spring and summer (1924) in field plots. These tests were necessarily confined to oats. Thirteen plots slightly larger than one square rod each were planted, arranged alternately in checker-board fashion with respect to checks and treated plots. There were six treated plots and seven not treated. All six of the treated plots received the first four applications of 90–10 dust, one plot received one additional dusting, and two received two additional applications. No attempt was made to apply any definite amount of dust per application, as the object was to determine only the effectiveness of sulfur in preventing rust infection. The applications were made just before rains as far as possible.

Artificial inoculation was made first by applying spore suspensions to the plants of all the plots by atomizer, and later by interplanting infected oats alike in two checks and in two treated plots. Heavy and general infection followed in the undusted plots.

A count of infected plants versus non-infected plants in plot 3 (check) and plot 4 (dusted) revealed phenomenal control. All plants in each plot were counted. In the dusted plot there was .03 per cent infected plants while in the checks (untreated) there was 74.35 per cent infected plants. An idea of the degree of control secured in all the plots can best be obtained by consulting the following table, for which counts of all the plants in five central rows of each plot are given.

Plot	No. of applications	Per cent of infected leaves
1a	0	93.35
1	7	0.08
2	0	42.50
3	0	99.14
4	7	0.00
5	5	0.26
6	0	73.02
7	0	98.40
8	4	21.23
9	4	14.60
10	0	90.30
11	0	90.00
12	4	12.60

In conclusion, the writer would point out that the results given are purely of preliminary value and not to be used as the basis for recommendations for the practical control of cereal rusts. More extensive field tests, not only on oats but also on wheat and rye, are under way this season.

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MISTLETOE IN THE LOWER BOLE OF INCENSE CEDAR

Willis W. Wagener

Irregular fusiform or barrel-shaped swellings are frequently found on the upper parts of the bole of Incense cedar, one of the important forest constituents of the California pine region. These swellings were first investigated by Meinecke[1] who found them to be caused by old established growths of the Incense cedar mistletoe, *Phoradendron libocedri* Howell. He pointed out that the development of the swellings was accompanied by a very remarkable change in the habit of the mistletoe. As the bark of the Incense cedar thickens, the green shoots of the mistletoe are pushed out with increasing difficulty until finally they become completely smothered and the mistletoe plant adapts itself to life as a true parasite in the tissues of the host tree. In this form the mistletoe attains ages much greater than known for other members of the genus or its Old World relative, *Viscum*. The resulting swellings develop to large size. Meinecke found one swelling in which the parasite had been present for 219 years. In point of size the largest measured by him was a barrel-shaped swelling 5 feet 8 inches in length.

Mistletoe is not the only cause of swellings on Incense cedar. As shown by Boyce,[2] enlargements of the trunk and branches may be due to the presence of the perennial mycelium of a rust, *Gymnosporangium blasdaleanum* (D. & H.) Kern. Due to their somewhat similar apparance, these may be confused with *Phoradendron* swellings from which green shoots are no longer produced, but the two can ordinarily be distinguished by their comparative size and shape.

The *Phoradendron* swellings are usually rather large and thick, while the swellings caused by the rust are smaller and very much narrower in outline. Typically, the latter are of a narrow spindle shape or, as described by Boyce, they resemble "the muscles of the upper arm when partially contracted."

Swellings from both causes occupy about the same range of position in the tree. The berries of the mistletoe are chiefly disseminated by birds which do not appear to frequent small Incense cedars. Should a berry by chance become lodged in a small tree, or in the shaded lower crown of a larger one, it evidently does not find conditions favorable for its establishment. As a rule, therefore, the Incense cedar mistletoe is found well up in older trees. *Gymnosporangium blasdaleanum* on the other hand very commonly occurs on small young trees but seems to die out as the trees grow older, as *Gymnosporangium* swellings are rare for the first log length on the boles of larger Incense cedars.

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[1] Meinecke, E. P. Parasitism of *Phoradendron juniperinum libocedri* Engelm. Proc. Soc. Am. For. 7: 35–41. 1912.

[2] Boyce, J. S. Perennial mycelium of *Gymnosporangium blasdaleanum*. Phytopath. 8: 161–162. 1918.

A swelling which varied markedly from the usual position for either *Phoradendron* or *Gymnosporangium* was found recently near Cow Creek on the Stanislaus National Forest, California, the same general locality from which Meinecke reported the *Phoradendron* swellings described by him. It occurred on an old Incense cedar, 50 inches in diameter inside bark at stump height, growing in a mixed coniferous stand at about 5,000 feet elevation. From a point 2 feet above ground up to 10.5 feet, one side of the trunk of this tree was swollen in an irregular spindle shape, with the region of greatest swelling at 5.3 feet from the ground. Here the swollen part measured 4.4 feet over the outside and occupied about 130° of the circumference of the tree. In the process of enlargement, the basal fluting, characteristic for the trunks of old Incense cedars, had been strongly accentuated, resulting in a division of the swelling by deep, longitudinal furrows into several ridges, widely expanded toward the outside over a rather narrow base. The location of the swelling suggested *Gymnosporangium blasdaleanum* as the casual organism, though it was considerably larger than any previously reported even for *Phoradendron libocedri* swellings. There were no surface indications to aid in a positive determination, but, upon the removal of a portion of bark from one of the ridges, rows of depressions in the wood were uncovered, from which projected the yellowish outer ends of living mistletoe sinkers, proving that the enlargement was not in fact due to *Gymnosporangium* but to the Incense cedar mistletoe. As far as is known this is the first reported case of the occurrence of *Phoradendron libocedri* so near the ground.

The mistletoe must have gained entrance while the host tree was still quite young. Under the assumption that the height at which entrance took place would be indicated approximately by the greatest degree of enlargement, a cross section cut was sawed through the thickest part of the swelling at 5.3 feet from the ground. The innermost sinker was found in the heartwood at about 7 inches from the center of the tree. The diameter of the tree at this point was thus 14 inches inside bark at the time the mistletoe appeared there. It would be impossible for a mistletoe seedling to penetrate the thick bark on so large a trunk. Both this fact and the absence of any signs of the bases of former mistletoe shoots in the wood at the cross section indicated that the cut had not passed through the point of initial entrance of the mistletoe. It is not unlikely that entrance was effected through the base of a nearby branch and that later the parasite gradually spread into the main trunk.

Allowing for several rings which could not be counted on account of rot in the center heartwood, the age of the tree at the cross section was about 448 years. Ring counts established the age of the mistletoe plant at this point at 409 years. Actually it must have been present for a number of years longer. Even 409 years is 200 years in excess of the greatest age recorded by Meinecke for the cases examined by him.

Phoradondrons, when young and vigorous, are known to have severely deleterious effects on their hosts, as is well illustrated in our Sierra forests by the spiked and broken tops found in White fir as the result of the presence of *Phoradendron pauciflorum*. Young plants of *Phoradendron libocedri* are not unlike other Phoradendrons in this respect, though their effect does not appear to be quite so severe as for some of the other species. As they grow older and gradually assume a truly parasitic character, the change appears to be accompanied by a general lowering of physiological requirements which results in little harm to the host. The merchantability of affected trees, however, is often seriously reduced.

In the parasitic form the mistletoe is apparently able to live on indefinitely, and there is no evidence that the natural life of the host is shortened by its presence. Incense cedar being a long-lived tree, *Phoradendron libocedri* is able to attain remarkably high ages. It would not be surprising if living specimens even older than the one described here were found.

OFFICE OF INVESTIGATIONS IN FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
SAN FRANCISCO, CALIF.

***Peridermium Harknessi* Moore in Western Yellow Pine Tops.**—*Peridermium harknessi* generally attacks seedlings and saplings most heavily, and seldom reaches above the lower branches of larger trees. An unusual case was found in 1924 on the Stanislaus National Forest, California, at an elevation of 5,000 feet, in which several good-sized Western Yellow pines were carrying the fungus in their tops. A group of four or five vigorously growing standards about 100 feet tall bore a number of galls in their crowns, above a height of 75 feet. The swellings ranged from 3 to 7 centimeters in diameter, and though most of them were dead, a few were still sporulating at the time of the observation early in October. At distances of 50 and 125 yards respectively from this group, stood two more infected trees, both about 150 feet tall. Each had a single sporulating gall on the leader within 3 feet of the tip. All of these pines were more or less aligned in the direction of the locally prevailing summer winds. About 200 yards away, though not strictly in line with the above, one other dead gall had been found earlier in the season. It was on a branch among the top litter of several felled trees which had formed part of a clump of suppressed individuals from 60 to 80 feet tall. The original height of the swelling could not be determined accurately, but it must have been supported at least 50 feet above ground.

The infected trees were located on a timber sale in a mature Sugar pine-Yellow pine stand, with a fairly rich understory of various-aged Yellow pine reproduction. The field work incidental to their discovery lasted for three and a half months from July to October, 1924. During this time every felled pine on a hundred acres of the logging operation was carefully examined. Constant attention also was given to the pathology of the young growth in the region. No more galls were observed and even *Castilleia* and *Pedicularis,* which were occasionally present, failed, after frequent examinations throughout the season, to reveal any signs of a *Cronartium.*

There is a bare possibility that the absence of uredinia and telia was due to the excessive drought of 1924, which may have hastened the fruiting period or inhibited it altogether. The apparent freedom of young Yellow pine from the disease rather seems to speak against the existence of an endemic condition of long standing based on local inter-relations between Scrophulariaceous hosts and pines. It seems far more probable that the infection, isolated within a large area and running in a well defined streak high above ground, had originated in the stand from chance sporidia or aeciospores carried in from the outside. Once established, the rust spread from pine to pine by direct aecial infection.—L. S. GILL.

OFFICE OF INVESTIGATIONS IN FOREST PATHOLOGY,
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SAN FRANCISCO, CAL.

AMERICAN PHYTOPATHOLOGICAL SOCIETY

CONSTITUTION

ARTICLE I

This Society shall be known as The American Phytopathological Society.

ARTICLE II

MEMBERSHIP

Sec. 1. The Society shall consist of members, and may include sustaining life members and patrons.

Sec. 2. The charter membership of this Society shall consist of the one hundred and thirty persons who accepted the invitation of the Organization Committee of October 25, 1909, to form the Society.

ARTICLE III

QUALIFICATIONS FOR MEMBERSHIP AND DUES

Sec. 1. All persons interested in the study of phytopathology, including the practical control of plant diseases, shall be eligible to membership.

Sec. 2. Each member shall pay annually such dues as the Society shall determine.

Sec. 3. Any member may become a sustaining life member by paying one hundred dollars in ten consecutive annual payments, and any person may become a patron upon the payment of one hundred dollars, and upon election shall have all the privileges of membership.

ARTICLE IV

ELECTION OF MEMBERS

Members may be elected at any regular meeting of the Society or by the Council during the interim. Applications for membership must be endorsed by at least one member of the Society.

ARTICLE V

OFFICERS

The officers of the Society shall consist of a President, Vice President, and Secretary-Treasurer. Their duties shall be those usually performed by such officers. The President and Vice President shall serve for one year and the Secretary-Treasurer for three years, or until their successors are elected, and the Council shall fill any vacancies occurring in the interim between elections.

The Council shall consist of the President, Vice President, Secretary-Treasurer, the retiring President, and the Chairman of the Board of Editors of the Journal of the Society, with two members elected, one each year, who shall serve for a term of two years, and one member elected annually by each Division. All action of the Council or officers must be authorized or approved by the Society.

ARTICLE VI

ELECTION OF OFFICERS

Officers shall be elected by a majority vote of the members present at the regular annual meeting.

ARTICLE VII

EDITORS AND COMMITTEES

The Editors of the official organ of the Society shall be selected by the Council subject to the approval of the Society. Temporary or standing committees may be appointed at the discretion of the Society.

ARTICLE VIII

MEETING

An annual meeting shall be held at such a time and place each year as the Council may select, and additional meetings, including special or local meetings, for the presentation of papers, may be arranged by the Council at its discretion.

ARTICLE IX

AMENDMENTS

This Constitution may be amended at any annual meeting by a three-fourths majority of all the members voting, notice of the proposed amendment having been sent to all the members at least one month previous to the meeting.

PROPOSED AMENDMENTS TO CONSTITUTION

The following amendments to the Constitution were submitted at the Washington Meeting and will be voted on at the Kansas City Meeting December 29, 1925, to January 1, 1926.

A. Change Article VI to read as follows:

ARTICLE VI

ELECTION OF OFFICERS

The Secretary-Treasurer shall send nomination ballots for offices to each member of the Society in time to allow all nominations to be returned not less than two months before the date of the annual meeting. The Council shall make nominations for any office when such nominations are wanting. The three candidates for each office receiving the highest number of nominating votes shall be placed upon a final ballot, which shall be sent to each member one month before the annual meeting. Votes shall be mailed to the Secretary-Treasurer and canvassed by the Council. A plurality vote shall elect.

B. Add the following article:

ARTICLE IX

DIVISIONS

Branch organizations or units within the Society known as Divisions may be established on a geographical basis, provided formal application setting forth the reasons for the establishment of the Division is made to, and approved by, the parent Society.

C. Amend Article V by inserting the following immediately before the last sentence of the Article:

The term of service of a council member from a Division shall commence at the beginning of the annual meeting of the Society next following his election by the Division.[1]

D. Change the number of the present Article IX (Amendments) to Article X.

STANDING RULES[2]

The rules under which the American Phytopathological Society operates are as follows:

PHYTOPATHOLOGY

1. The official publication of the Society shall be *Phytopathology*.

Officers

a. The officers of *Phytopathology* shall be an editor-in-chief, term three years; two editors, terms three years; twelve associate editors, terms three years, four selected each year; business manager, term one year; and advertising manager, term one year.

Selection of Officers

b. The editor-in-chief and the business manager shall be selected by the Council and approved by the Society. The editors and the twelve associate editors shall be selected by the Council in consultation with the editor-in-chief and approved by the Society. The advertising manager shall be selected by the Council in consultation with the business manager and approved by the Society.

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[1] Note that Article V, paragraph 2, was amended at the Baltimore Meeting by adding at the end of the first sentence the words "for a term of two years and one member elected annually by each Division." (Phytopath. 9: 1-86. 1919.)

[2] Submitted by Committee on Codification of Rules and adopted by the Society at the Washington meeting, January [illegible]25.

Subscriptions, Back Numbers

c. Subscriptions to *Phytopathology* for institutions and non-members shall be $5 per year in the United States and dependencies, Mexco, and Cuba; Canada $5.25; other countries $5.50. The price of current single numbers shall be 50 cents. Back volumes with the exception of volumes 4, 5, and 6 may be obtained unbound at $6 per volume, postage paid. Separate copies will not be sold except in cases where the volumes are already broken, the price of such copies shall be $1 for each number in volumes one to seven inclusive, and 50 cents each for subsequent years. Requests to supply lost copies of the journal without charge must be made within sixty days from date of issue.

DUES

2. The annual dues for each regular member including subscription to *Phytopathology* shall be $4 per year, payable on December 20. The business manager of *Phytopathology* shall discontinue sending the journal to any members whose dues have not been received by December 20.

PAPERS, ABSTRACTS

3. Members desiring to present papers at the annual meeting must furnish to the secretary carefully prepared abstracts presenting as clearly and concisely as possible the substance and conclusions of the papers, these abstracts to embody definite results and not to exceed two hundred words in length.

Date Due

a. The secretary is authorized to refuse abstracts received by him after the date on which they are due (Nov. 15). Members are [illegible]quested not to submit titles or abstracts unless they intend to be present at the meetings.

Number of b. No member shall be permitted to present more than two papers at any one meeting except by invitation, and in case of joint authorship the paper shall be charged to the author presenting it.

Editing of c. All abstracts shall be submitted to an editorial committee of at least three selected by the editor-in-chief of *Phytopathology* and this committee will edit the abstracts in the same manner as original articles published in *Phytopathology*.

PROGRAMS 4. The program for the annual meeting shall be in charge of a program committee consisting of the president, secretary, and chairman of the Advisory Board. To relieve congestion the program committtee is authorized to schedule simultaneous sessions when necessary.

DIVISIONS 5. The following provisions shall govern the organization and regulation of Divisions of the Society.

Name of a. Divisions shall use the name of the parent society with the appropriate geographical term, for example, American Phytopathological Society, Pacific Division.

Membership b. Divisions shall elect to full membership only members of the American Phytopathological Society, but each Division may elect associate members under such rules as it may adopt.

Publication c. The proceedings of Divisions shall be printed in *Phytopathology*. The preliminary abstracts of the Division meetings may, at the discretion of these Divisions, be printed in *Phytopathology* under the same rules that govern publication of abstracts of the general society. This rule, however, shall not be interpreted as limiting the present right of the Editorial Board of *Phytopathology* to define the character and amount of any manuscript for publication.

Meetings of d. Whenever the American Phytopathological Society meets within the territory of a Division, the Division shall merge its program with that of the parent Society. The scientific sessions of such a meeting shall be presided over alternately by the president of the American Phytopathological Society and the president of the Division. Business sessions may be independent.

Constitution of

e. The constitution or articles of organization of all Divisions shall contain a provision or provisions ratifying the above rules. The constitution of all Divisions shall contain nothing in conflict with the constitution of the American Phytopathological Society. With the exceptions defined by the above rules the Divisions shall enjoy complete autonomy.

SECRETARY'S EXPENSES

6. Unless otherwise ordered the secretary is authorized to attend the annual meetings of the Society at the Society's expense.

AUDITING COMMITTEE

7. At each annual meeting the president shall appoint an auditing committee to audit the accounts of the secretary-treasurer and the business manager of *Phytopathology*.

ADVISORY BOARD

8. There shall be a permanent committee of the Society called the Advisory Board working under the direction of and reporting to the Council. The personnel of this board shall be eight members appointed by the Council, two each for periods of one, two, and three years, and thereafter two each year for a three-year term, these members to represent the United States Department of Agriculture and the following regions—Northeast, South, Middle West, Pacific Coast, and Canada; and two members at large for terms of three years. The chairman of the Board shall be elected annually by the Board.

Duties of

The duties of the Board shall be:

a. To represent the American Phytopathological Society before the National Research Council. The chairman of the Board shall be the Society's representative on the Division of

Biology and Agriculture of the National Research Council.

b. To function as the committee on cooperation with the Crop Protection Institute and the Tropical Research Foundation.

c. To arrange for conferences of groups of workers on related subjects at the annual meetings or elsewhere.

d. To confer with workers in related fields, i e., entomology, genetics, horticulture, and agronomy, to promote joint efforts on our common problems.

e. To promote international relations in phytopathology.

f. To take up such other problems as the Board may find desirable, subject to the approval of the Council.

UNION OF BIOLOGICAL SOCIETIES

9. The society shall participate in the work of the Union of American Biological Societies and designates its secretary and editor-in-chief of *Phytopathology* as its representatives unless otherwise voted.

BOTANICAL ABSTRACTS

10. The Society shall elect two representatives on the Board of Control of *Botanical Abstracts* for terms of four years each, one being chosen every other year.

CROP PROTECTION INSTITUTE

11. Representation on the Board of Governors of the Crop Protection Institute shall be provided for by three trustees, with three year terms, one selected each year, these trustees to be chosen by the Council, with the proviso that one of them shall also be a member of the Advisory Board of the Society.

OTHER REPRESENTATIVES

12. The following shall be selected by the Council and approved by the Society: two representatives on the Council of American Association for the Advancement of Science for one year terms; one trustee on the Tropical Research Foundation for a five year term, and one member of the Editorial Board of the American Journal of Botany for a three-year term.

AMENDMENTS 13. These rules may be amended by a majority vote of the members voting at any regular meeting of the Society.

LIST OF PATRONS AND MEMBERS

SEPTEMBER 1, 1925.

PATRONS

CAPP, S. B., P. O. Box 2054, Philadelphia, Pennsylvania.

DAVIS, J. J., Curator of Herbarium, University of Wisconsin, Madison, Wisconsin.

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MEMBERS

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ARTSCHWAGER, E. F., Associate Pathologist, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

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* Life sustaining members paying currently.

† Life sustaining members who are paid up for life.

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In Memoriam

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Bell, A. T.	1914	1919
Bessey, Charles E. (Charter Member,	1908	1915
Carleton, Mark H. (Charter Member)	1908	1925
Clarkin, J. H.	1911	1913
Crittenden, Charles G.	1915	1918
Durand, E. J.	1911	1922
Elliot, John A.	1916	1923
Farlow, W. G. (Charter Member)	1908	1919
Halsted, Byron D. (Charter Member)	1908	1918
Henderson, Martin P.	1912	1923
Jones, W. Ralph	1911	1915
Kurtzweil, C. E.	1919	1921
Laird, K. B.	1915	1919
McMurran, S. M.	1911	1920
Martin, W. A.	1914	1916
Oberly, Eunice R.	1919	1921
Pond, R. H.	1911	1911
Puttick, G. F.	1919	1923
Ravn, F. Kölpin	1911	1920
Riddle, Lincoln W.	1919	1921
Roark, E. W.	1914	1918
Rosenbaum, Joseph	1911	1925
Selby, A. D. (Charter Member)	1908	1924
Stewart, V. B. (Charter Member)	1908	1918
Taylor, Rose M.	1918	1918
Thomas, M. B. (Charter Member)	1908	1912
Vasey, H. E.	1917	1918
Werkenthin, Fred C.	1915	1922
Young, Youngen	1914	1916

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NEW SEED DISINFECTANTS FOR THE CONTROL OF BUNT OF WHEAT AND THE SMUTS OF OATS AND BARLEY

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WITH PLATES XXV TO XXVIII

INTRODUCTION

The subject of seed treatment is one on which a large amount of investigation has been done. Yet it is a subject decidedly in need of further thorough and critical study. There is a striking need for disinfectants which will control seed-borne diseases and at the same time cause no injury to the treated seed. This demand has led scientific investigators and commercial organizations in the past to develop and study numerous chemical substances and compounds with the hope of finding something satisfactory. Of the several preparations tested, the great majority have proved worthless; others have limited use; while a few, including formaldehyde and copper-sulphate-lime, have proved of sufficient value to bring them into extensive use. However, seed treatment has not been practiced as generally as the needs would seem to demand. This is true in the case of certain of the cereal smuts which are comparatively easily controlled. One of the reasons for this lies in the fact that more or less seed injury often is caused by the generally recommended formaldehyde and copper-sulphate-lime treatments. Too often these complaints of seed injury have been explained away on the basis of improper preparation or handling of the treatment. It is now known that many factors may influence the effects of the treatment on the seed. Among the more important of these factors, not including variations that may occur in the material, its preparation and its application, are the kind of seed, the particular variety treated, the conditions under which the seed is grown and subsequently handled, and the local soil and weather conditions existing where the seed is sown after treatment. As these various limiting factors have made it almost impossible, even for the expert, to obtain satisfactory results with the treatments in use, there has been a keener interest in the search for disinfectants which are not injurious to the seed, at least within reasonable limits, as to strength of material and time of application. During the past decade investigations of this kind by both scientists and commercial organizations have become very intensive. Out of these studies a few materials of importance and promise have evolved. The more important of these are copper carbonate, the effectiveness of which is thoroughly established for the control of bunt in wheat, and some of the organic mercury compounds.

The object of these investigations has been to test these new compounds in comparison with the standard treatments, with the hope of finding something of greater value for the control of cereal smuts and other seed-borne diseases. The data presented herein necessarily are fragmentary, owing to the fact that some of the materials were not available during all years, while others were discarded as soon as they were found valueless.

HISTORY

The literature dealing with the new materials, especially the mercury compounds, is becoming so voluminous that it is not desirable to attempt to review all of it in this paper. Chlorphenol-mercury, the basis for such commercial preparations as Uspulun, Chlorophol, and Semesan, was used in Germany (9) for the control of bunt in wheat as early as 1912, if not earlier. The favorable results reported by Riehm at this time and one year later (10) no doubt led to the commercializing of this material under the trade name "Uspulun." In 1914, Remy and Vasters (8) published a rather comprehensive paper on the use of Uspulun. They recommended it for the control of certain seed-borne and foliage diseases as well as for the control of aphis. Since the appearance of these papers, numerous reports have been published in Germany and other countries on the use of Uspulun and several other related and unrelated compounds. As is to be expected, some conflicting evidence has been presented but, on the whole, the reports indicate that the chlorphenol-mercury compounds are valuable. In 1920 Germisan (1), another organic mercury preparation (probably cyan-mercury-cresol), appeared on the market in Germany. The reports concerning Germisan have about the same status as those dealing with Uspulun, indicating that it merits further consideration in our program of investigation in this country. Various other German preparations have appeared on the market, but few of them seem to have merit (3). Among these few are preparations of copper-arsenic, ortho-nitro-phenol-mercury-sulphate and mercury-oxycyanide. Of the mercury materials prepared in this country, Semesan, Chlorophol, and [illegible] No. 620 have proved to be of value. The organic mercury compounds are more or less insoluble in water but are soluble in alkaline solutions. For this reason the commercial preparations contain alkalis.

During the past four years considerable interest has been shown in these organic mercury compounds in this country. Heald and Smith (4) found that Chlorophol would control bunt in wheat but they did not consider it as satisfactory as copper carbonate. Tisdale, Taylor, and Griffiths (11), in preliminary experiments, obtained excellent results with Chlorophol in the control of barley smuts. Since that time there has been published a number of reports on the use of the organic mercury materials as well as on other compounds and mixtures for the control of various seed-borne and soil-inhabiting fungi. Among these are both solutions and dusts.

The value of copper-carbonate dust in the control of bunt of wheat is of more recent discovery than the use of chlorphenol-mercury as a disinfectant. Darnell-Smith (2) of Australia reported the successful use of copper carbonate in 1917. It was first used in this country by Mackie and Briggs (6, 7) in 1920 for the control of bunt. Since its introduction it has become the most popular treatment for the control of bunt of wheat. The reasons for this popularity are its effectiveness, ease of application, elimination of soaking, lack of seed injury, and low cost. Copper carbonate has its limitations, however. It has not proved satisfactory for the control of the smuts of oats and barley, as will be shown by the results reported in this paper.

PRESENT INVESTIGATIONS

As previously stated, these investigations were undertaken with the chief object of testing new materials in the hope that something more satisfactory than the old standard treatments might be discovered. These materials were tested for the control of bunt of wheat and smuts of oats and barley. These investigations covered a period of four years, beginning in the spring of 1921, when hull-less oats were treated with Chlorophol, and extending through the crop season of 1924. For all of these experiments naturally infested seed was obtained when it could possibly be found and, in all cases except a few uninoculated untreated controls, the seed was thoroughly inoculated with viable smut spores before treating, whether it was naturally infested or not. The barley seed was inoculated with the spores of covered smut only. The loose smut came from natural infestation. In the fall of 1923 the oat and barley seed was run through a scarifying machine with the hope of rendering the inoculation more effective, as the removal of the hulls of barley had previously been found to render the seedlings more susceptible to infection (12).

In the fall of 1921 seed of Purplestraw wheat, Winter Turf oat, and Tennessee Winter and Han River barleys was treated with Chlorophol, copper carbonate, and some of the old standard treatments, and sown in fortieth-acre plats on Arlington Farm, Virginia. Uniform machine-threshed seed of pure varieties grown on Arlington Farm the previous year was used for these experiments. In order to obtain accurate germination records on all treated seeds, two packets of 500 seeds each were counted out from each of the lots of seed, both treated and untreated, which were sown in the fortieth-acre plats. These counted seeds were sown in rod rows, 500 seeds to two rod rows, in soil as nearly comparable with the soil in the fortieth-acre plats as could be had. The rod-row sowings were duplicated with the second packet of counted seeds in each case. After their emergence the seedlings were counted and the percentages of emergence calculated from these figures. The emergence records from these duplicate plats of 500 seeds each should give a fair idea of the relative effects of these materials on the seed.

The smut records were obtained from the fortieth-acre plats by counting a given number of heads at regularly spaced intervals throughout the plats and recording the number of smutted heads. In cases where the percentages were low, less than one per cent, the smutted heads in the entire plat were counted. In the case of oats sown in the spring, the seed was counted and sown in rod rows. No fortieth-acre plats were sown and no attempt was made to obtain yield data. Only emergence counts and smut records were taken.

ROD-ROW EXPERIMENTS

As it was found to be impossible to run all of the tests in fortieth-acre plats, the materials were given a preliminary test in rod-row sowings. In these preliminary tests these compounds generally were used in several strengths, as many German investigators had reported that the strengths of solution recommended by the producers were too weak. The treatments which gave good results in the rod-row tests were later employed in the experiments in fortieth-acre plats.

The first of the chlorphenol-mercury compounds tested was Chlorophol. The solution was prepared by dissolving the Chlorophol in warm water, 3 grams in each liter of solution to be used. The seed was soaked for one hour and spread to dry before sowing. This treatment was applied to hull-less oats sown in rod rows in the spring of 1921, on Arlington Farm, Virginia, and at Manhattan, Kansas.[1] Both the loose and covered smuts were satisfactorily controlled with Chlorophol and there were no injurious effects on germination and plant growth, while formaldehyde (1:320, 10 min. soak) caused severe seed injury. A repetition of this experiment in 1922 was not so successful, due, evidently, to reinfestation of the seed.

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[1] The writers are indebted to Mr. C. O. [illegible] for sowing the seed and taking the notes at Manhattan.

TABLE 1.—*Effects of certain organic mercury compounds on the rate of emergence of Winter Turf oat seedlings from seed inoculated with Ustilago avenae, treated on January 11, 1922, and sown in soil on greenhouse benches at Arlington Farm, Rosslyn, Virginia, on the following day*

Treatment			Total number of seedlings emerged, on successive dates, from 400 seeds sown							Total emergence
Material	Strength of solution	Duration of soak	January					February		
			20	21	23	24	26	2	10	
	Pct.	Hours								Pct.
Dupont S.D. No. 12, salt	.1	1	154	246	320	335	356	384	386	96.50
Dupont S.D. No. 12, salt	.3	1	114	192	262	285	284	351	377	94.25
Chlorophol	.1	1	200	302	354	368	373	380	383	95.75
Chlorophol	.3	1	143	250	344	361	371	386	377	94.25
Untreated, inoculated			202	293	358	362	368	375	372	93.00
Untreated, uninoculated			155	255	342	345	361	371	369	92.25
Uspulun	.1	1	192	287	350	359	371	373	375	93.75
Uspulun	.3	1	114	240	331	348	365	377	377	94.25
Dupont S.D. No. 12, solution, 3–400			12	54	145	156	173	271	341	85.25
Dupont S.D. No. 12, solution, 1–400			134	222	293	309	326	368	373	93.25
Chlorophol	.3	1	254[a]	323	363	364	367	380	378	94.50
Dupont S.D. No. 12, dusted			35	65	137	146	151	250	271	67.75

[a] Treated November 25, 1921, and dried; sown January 12, 1922.

In the fall of 1921 seed of Winter Turf oat and Han River and Tennessee Winter barleys were treated with Chlorophol, copper carbonate and some of the standard treatments, and sown in rod rows (for emergence records) and in fortieth-acre plats to obtain smut control and yield data. In the fortieth-acre plats the seedlings grown from the Chlorophol-treated seed were much more vigorous than those from untreated seed or from seed treated with formaldehyde. The plats with seedlings from Chlorophol-treated seed could be distinguished easily by persons knowing nothing of the treatments. This led to a consideration of the possibility that there might be stimulation, as the German investigators had claimed. There certainly was a striking and easily measurable difference in the development of the seedlings. The question arose as to whether this was due to earlier germination of the seed or to a more rapid growth of the seedlings, or possibly to both. It seemed entirely possible that the seed coat was rendered more permeable and that this might have hastened germination by aiding the process of oxidation. It also seemed probable that, through having been effectively disinfected, the seed was freed from saprophytic as well as parasitic organisms and the seedling enabled to develop a clean and more efficient root system, and consequently more vigorous growth.

In a preliminary experiment in the greenhouse in the winter of 1921, it was found that both Chlorophol and Uspulun had a beneficial effect on the germination of Winter Turf oat and Purplestraw wheat from machine-threshed seed grown the previous season on Arlington Farm. In each case emergence was increased, the increases ranging from 4 per cent to 7.5 per cent. The seedlings also were more vigorous. In 1922 an effort was made to determine the rate of emergence by treating seed of Winter Turf oat with the mercury compounds then available and sowing them in carefully prepared soil in greenhouse benches. These materials were prepared when used in solution by weighing out 1, 2, or 3 grams, according to strength desired, for each liter of solution to be used, and then dissolving in the desired quantity of warm water. The seed was placed in the solution in cheesecloth bags and soaked for the desired time, and then spread to dry before sowing. Where dusts were used they were applied to the measured grain at the proper rate, generally 2 to 3 ounces per bushel, and the grain was then shaken in a jar or closed can or box until each kernel was thoroughly covered with dust. These experiments were conducted in January and February, 1922. In both experiments the seed was treated one day previous to sowing.

The results of these experiments, as given in tables 1 and 2, show that some of these materials had a tendency to hasten germination slightly. This is particularly true of Chlorophol in table 1, line 11, where the seed

TABLE 2.—*Effects of certain organic mercury compounds on the rate of emergence of Winter Turf oat seedlings from seed inoculated with Ustilago avenae, treated on January 25, 1922, and sown in soil on greenhouse benches at Arlington Farm, Rosslyn, Virginia, the following day*

Treatment			Total number of seedlings emerged, on successive dates, from 400 seeds sown						Total emergence
Material	Strength of solution	Duration of soak	February						
			2	4	6	9	14	18	
	Pct.	Hours							Pct.
Corona No. 10	.2	1	7	132	301	375	385	387	96.75
Corona No. 10	.1	1	31	233	350	383	384	387	96.75
Dupont S.D. No.12, dry	.1	1	8	208	311	344	359	366	91.50
Corona No. 620	.2	1	6	184	308	360	367	374	93.50
Corona No. 620	.1	1	0	265	352	381	383	383	95.75
Corona No. 30	.2	1	8	241	348	368	379	379	94.75
Corona No. 30	.1	1	11	245	364	387	388	386	96.50
Untreated, inoc.			4	153	328	367	371	369	92.25
Untreated, uninoc.			12	183	322	370	375	373	93.25
Corona No. 640–S, dusted			1	36	242	358	378	378	94.50
Corona No. 50	.2	1	1	19	53	183	248	271	67.75
Corona No. 50	.1	1	2	24	93	253	292	319	79.75
Chlorophol	.3	1	21	258	330	374	385	385	96.25
Uspulun	.3	1	1	209	332	375	384	385	96.25

was treated with a .3 per cent solution on November 11, 1921, and sown January 12, 1922, after it was thoroughly dry. These seedlings were noticeably further advanced when the first emergence counts were made than were those from untreated seed, although the final records for emergence show the percentages to be but little different. In table 2, the records of February 2 show emergence to be slightly better from seed treated with Corona No. 10 (.1 per cent, 1 hour soak) and Chlorophol (.3 per cent, 1 hour soak), while all other materials used seemed to retard emergence. On February 4, most of these treatments showed better results than did the untreated seed. In both tables 1 and 2, seed treated with these materials, with the exception of Dupont S.D. No. 12 (solution 1–400), Dupont S.D. No. 12 (dusted), and Corona No. 50, finally emerged about as well as or better than did the seedlings from untreated seed.

In further preliminary field tests on spring-sown Kherson oat in 1922 and 1923, a number of compounds were used in different strengths and the seed was soaked for different periods of time. All of these materials were not used in both years because, as previously stated, some of them were available in only one year and others were eliminated because proving unsatisfactory after the first year's test. Time and space were too limited to continue with materials of no promise. An important factor which renders results with such materials uncertain is the question as to whether the chemical composition of any given compound has remained the same. Some samples of certain of them have contained dyes, while other samples of the same compound have not. There also has appeared to be a difference in the rate at which different samples under the same name will dissolve in water at approximately the same temperature. However, the results have been fairly consistent with some of these compounds.

After the first year's experiments, previously described, warm water was not used in dissolving the organic mercury compounds. They were weighed out and placed in the desired quantities of tap water and dissolved by thorough stirring. Some few of the materials were furnished by the manufacturers with rather complicated directions for their preparation and application. Since none of these have proved satisfactory it will not be necessary to give these directions. After treating the seed it was thoroughly dried and sown as soon after drying as possible. The results of the tests with spring-sown Kherson oat are given in table 3. Very few of the materials listed in this table are being manufactured. However, the table will give some idea of the number of materials tested and the reasons for eliminating many of them.

The data in table 3 show that several of these materials gave fairly good results in the control of oat smut, [illegible] some of them had a decidedly

TABLE 3.—*Effects of various seed disinfectants on the emergence and percentages of smut in spring sown Kherson oat after the seed was inoculated with spores of loose and covered smuts, and sown on Arlington Farm, Virginia, in 1922 and 1923*

Treatment			Average percentages					
Material	Strength of solution	Duration of soak	1922		1923		Two years	
			Germination	Smut	Germination	Smut	Germination	Smut
	Pct.	Hours						
Untreated, inoc.			60.0	10.60	85.8	3.75	72.90	7.17
Chlorophol	.3	1	72.2	1.55	91.7	0.0	81.95	0.77
Chlorophol	.3	½	71.7	0.15	92.0	0.0	81.85	0.07
Chlorophol	.3	¼	69.6	0.15				
Chlorophol	.2	1	72.8	0.0	90.2	0.0	81.50	0.0
Chlorophol	.2	½	72.4	0.40	90.7	0.15	81.55	0.27
Chlorophol	.2	¼	67.9	0.54				
Chlorophol	.1	1	68.7	0.14				
Chlorophol	.1	½	67.1	0.27				
Chlorophol	.1	¼	64.9	0.41				
Chlorophol	.05	1	71.2	0.25				
Chlorophol	.05	½	71.2	2.46				
Chlorophol	.05	¼	60.8	1.20				
Untreated, uninoc.			63.0	1.34				
Chlorophol	.06 (spray)		64.0	7.56				
Corona No. 10	.2	1	69.3	1.71				
Corona No. 10	.2	½	64.8	1.53				
Corona No. 10	.2	¼	63.7	0.83				
Corona No. 10	.1	1	68.9	2.15				
Corona No. 10	.1	½	69.4	1.73				
Corona No. 10	.1	¼	68.0	4.38				
Corona No. 10	.05	1	65.0	1.50				
Corona No. 10	.05	½	63.3	3.11				
Corona No. 10	.05	¼	67.1	0.15				

TABLE 3.—(*Continued*)

Treatment			Average percentages					
Material	Strength of solution	Duration of soak	1922		1923		Two years	
			Germination	Smut	Germination	Smut	Germination	Smut
	Pct.	Hours						
Corona No. 10	.6 (spray)	......	63.5	5.37	......	......	......	......
Untreated, uninoc.	......	......	51.6	0.35	......	......	......	......
Untreated, inoc.	......	......	56.7	10.07	87.9	2.7	......	......
Semesan	.3	½	......	......	92.7	0.4	......	......
Semesan	.2	1	......	......	92.5	1.5	......	......
Semesan	.2	½	......	......	92.9	1.25	......	......
Semesan	.1	1	......	......	92.1	1.55	......	......
Germisan	.3	1	......	......	93.0	0.0	......	......
Germisan	.3	½	......	......	90.3	0.0	......	......
Germisan	.2	1	......	......	94.0	0.0	......	......
Germisan	.2	½	......	......	92.9	0.05	......	......
Germisan	.1	1	......	......	93.4	0.1	......	......
Germisan	.1	½	......	......	94.6	0.05	......	......
Tillantin B	.2	1	......	......	86.8	0.3	......	......
Tillantin B	.2	½	......	......	88.5	0.45	......	......
Tillantin C	.2	1	......	......	90.7	0.05	......	......
Tillantin C	.2	½	......	......	87.7	0.2	......	......
Untreated, inoc.	......	......	......	......	84.7	4.35	......	......
Corona No. 620	.2	1	62.9	0.30	88.3	0.05	75.60	0.01
Corona No. 620	.2	½	57.7	0.0	85.5	1.02	71.60	0.51
Corona No. 620	.2	¼	64.1	0.0	85.9	1.05	75.00	0.57
Corona No. 620	.1	1	73.2	0.0	86.1	0.77	79.65	0.38
Corona No. 620	.1	½	76.1	0.64	84.8	1.85	80.45	1.24
Corona No. 620	.1	¼	76.6	0.39	89.3	1.25	82.95	0.82
Corona No. 620	.05	1	71.2	0.13	89.9	1.65	80.55	0.89

TABLE 3.—(*Continued*)

Treatment			Average percentages					
Material	Strength of solution	Duration of soak	1922		1923		Two years	
			Germination	Smut	Germination	Smut	Germination	Smut
	Pct.	Hours						
Corona No. 620	.05	½	70.6	0.41	90.5	0.93	80.55	0.67
Corona No. 620	.05	¼	77.5	0.39	89.3	1.17	83.40	0.78
Corona No. 620	.6 (spray)	......	72.0	1.66	......	......	......	......
Corona No. 620, impure	.2	1	72.7	0.0	......	......	......	......
Corona No. 620, impure	.2	½	67.8	0.87	......	......	......	......
Corona No. 620, impure	.2	¼	73.5	0.0	......	......	......	......
Uspulun	.3	1	72.5	0.0	......	......	......	......
Uspulun	.3	½	74.8	0.0	......	......	......	......
Uspulun	.3	¼	70.6	0.94	......	......	......	......
Uspulun	.1	1	70.4	0.15	......	......	......	......
Uspulun	.1	½	75.9	1.05	......	......	......	......
Uspulun	.1	¼	72.6	1.40	......	......	......	......
Untreated, uninoc.	......	......	63.5	0.15	......	......	......	......
Untreated, inoc.	......	......	57.3	5.59	......	......	......	......
Formaldehyde, 1: 320	......	⅙	56.0	0.0	73.7	0.18	64.85	0.09
Formaldehyde, 1: 1 (spray)	......	......	21.8	0.0	......	......	......	......
Formaldehyde, 1: 320	......	⅙ wash in water	......	......	76.3	0.98	......	......
Formaldehyde, 1: 320	......	⅙ wash-lime water	......	......	83.1	0.31	......	......
Formaldehyde, 1: 320	......	½	......	......	30.0	0.0	......	......
Formaldehyde, 1: 320	......	½ wash in water	......	......	75.4	trace	......	......
Formaldehyde, 1: 320	......	½ wash-lime water	......	......	78.1	0.0	......	......

TABLE 3.—(*Continued*)

Treatment			Average percentages					
Material	Strength of solution	Duration of soak	1922		1923		Two years	
			Germination	Smut	Germination	Smut	Germination	Smut
	Pct.	Hours						
Formaldehyde, 1: 320	......	1	......	......	27.0	0.0	......	......
Formaldehyde, 1: 320	......	1 wash in water	......	......	66.0	0.0	......	......
Formaldehyde, 1: 320	......	1 wash-lime water	......	......	72.9	0.18	......	......
Fertilisa, soak	......	5	59.8	0.0	......	......	......	......
Fertilisa, soak	......	3	61.5	0.08	......	......	......	......
Kalimat	.3	½	......	......	78.2	0.0	......	......
Pythal	.75	½	......	......	91.4	0.43	......	......
Untreated, inoc.	......	..	......	......	86.5	5.7	......	......
Dupont S.D. No. 12, powder	.1	1	57.1	0.71	......	......	......	......
Dupont S.D. No. 12, powder	.1	½	55.9	0.52	......	......	......	......
Dupont S.D. No. 12, liquid, 1:400	......	1	68.1	0.56	......	......	......	......
Dupont S.D. No. 12, liquid, 1:400	......	½	57.8	1.96	......	......	......	......
Dupont S.D. No. 1	.3	1	65.5	0.33	......	......	......	......
Dupont S.D. No. 1	.1	1	70.4	0.41	......	......	......	......
Dupont S.D. No. 2	.3	1	65.4	0.45	......	......	......	......
Dupont S.D. No. 2	.1	1	63.1	1.40	......	......	......	......
Untreated, uninoc.	......	—	51.0	0.75	......	......	......	......
Untreated, inoc.	......	..	52.8	15.77	81.6	5.50	67.20	10.63
Corona No. 40F, coarse powder	......	dust	63.1	1.73	......	......	......	......
Corona No. 40F, with inert	......	dust	58.1	3.23	......	......	......	......
Corona No. 640–S	.	dust	54.0	1.20	86.9	1.5	70.45	1.35
Seed-O-San, pink dust	..		53.4	7.09	88.5	2.75	70.95	4.92
Seed-O-San, white dust	......	..	......	......	83.5	1.5	......	......

TABLE 3.—(*Continued*)

Treatment			Average percentages					
Material	Strength of solution	Duration of soak	1922		1923		Two years	
			Germination	Smut	Germination	Smut	Germination	Smut
	Pct.	Hours						
Copper Carbonate C.P., 2 oz. per bu.		dust	47.2	0.57	82.3	1.45	64.75	1.01
Corona Coppercarb		dust	54.4	1.39				
Coppercarb, 2 parts, to Dupont S.D. No. 2, 1 part		dust	56.8	1.76				
Dupont Dust Disinfectant, No. 2		dust			83.8	1.65		
Dupont Dust Disinfectant, No. 6		dust			86.9	1.40		
Untreated, uninoc.			55.5	0.55				
Untreated, inoc.			55.2	10.48	81.6	5.5	68.4	7.99

beneficial effect on germination of the seed. These differences easily could be seen in the field a few days after the seedlings had emerged, and often were maintained throughout the life of the plants, as is indicated by the yields from some of the fortieth-acre plats (Table 6). This difference could not always be seen, however. In the Winter Turf oat in 1923 there was practically no discernible difference in the plants at any stage of development. All of the plats were so poor, however, that no attempt was made to obtain yield data.

In a further test of some of these treatments on Cusado barley and Winter Turf oat sown in the fall of 1922 on Arlington Farm, germination records were obtained from duplicate sowings of 500 seeds each. Smut records, the number of smutted heads per rod row, were obtained on the barley the following spring, but no smut records were obtained on the oats because very little smut developed, even in the plants from untreated seed. In the fortieth-acre plats, with the same treatments, none of these compounds completely controlled either of the two oat smuts. There was only a trace of smut in the check plats, however. None of the oats from treated seed was entirely smut-free. The results of these rod-row tests of oats and barley are shown in table 4.

The data in table 4 show that some of these materials were more effective than formaldehyde (1:320, 10 min.) for the control of both smuts of barley, and in many cases germination both of oats and barley was improved rather than injured, in contrast to results from using formaldehyde.

EXPERIMENTS IN FORTIETH-ACRE PLATS

Only the treatments which in rod-row experiments showed no harmful effects to germination and at the same time controlled the smuts were used for treating seed for the fortieth-acre plats. The preparation and application of the treatments were the same as described under rod-row experiments. Seedling-emergence records and subsequent smut and yield records were obtained from these plats.

Bunt of wheat. Comparatively few of these liquid treatments were used in these experiments for the control of bunt for the reason that copper carbonate had proved so satisfactory. As copper carbonate is a dust and so easy to apply, it hardly seemed worth while to use solutions in the hope of finding something so much better as to replace the dust. After the first year some of the liquids were discarded. In the third year only one solution, copper-sulphate-lime, was used. Such dusts as were available and seemed to be promising were used in these tests. Table 5 gives the germination and bunt percentages and the [illegible] yields in bushels of Purplestraw wheat treated with these various co[illegible]. The 1922 and 1923 figures

TABLE 4.—*Effects of various seed disinfectants on the germination of Cusado barley and Winter Turf oat, and on the control of the smuts in barley. Seed sown on October 4 and 5, 1922, Arlington Farm, Virginia.*

Treatment			Cusado barley									Winter Turf oat		
			Plat 1			Plat 2			Average			Germination		
Material	Strength of solution	Duration of soak	Germination	Heads in rod row: Covered smut	Heads in rod row: Loose smut	Germination	Heads in rod row: Covered smut	Heads in rod row: Loose smut	Germination	Heads in rod row: Covered smut	Heads in rod row: Loose smut	Plat 1	Plat 2	Average
	Pct.	Hour	Pct.			Pct.			Pct.			Pct.	Pct.	Pct.
Untreated, inoc.			74.8	55	7				74.80	55.0	7			
Uspulun	.3	1	76.6	0	0	89.0	0	0	82.80	0	0	73.8	76.0	74.90
Uspulun	.3	½	61.2	0	0	84.0	1	0	72.60	.50	0	76.0	77.0	76.50
Uspulun	.15	1	79.2	0	0	83.0	4	0	81.10	2	0	73.6	78.0	75.80
Germisan	.3	1	82.4	0	0	83.0	0	0	82.70	0	0	75.4	73.0	74.20
Germisan	.3	½	80.8	2	0	86.0	2	0	83.40	2	0	82.0	81.0	81.50
Germisan	.15	1	82.8	8	0	85.0	8	1	83.90	8	.50	83.6	85.0	84.30
Untreated, inoc.			77.4	47	22	81.0	62	1	79.20	54.50	11.50	83.2	90.0	86.60
Chlorophol	.3	1	79.8	0	0	83.0	0	0	81.40	0	0	84.4	76.0	80.20
Chlorophol	.3	½	78.6	0	0	87.0	0	0	82.80	0	0	85.6	86.0	85.80
Chlorophol	.15	1	87.6	0	0	79.0	0	0	83.30	0	0	82.6	87.0	84.80
Semesan	.3	1	86.6	0	0	76.0	0	0	81.30	0	0	83.4	83.0	83.20
Semesan	.3	¼	84.8	1	0	73.0	5	1	78.90	3.00	.50	74.6	82.0	78.30
Semesan	.2	1	82.0	41	3	77.0	26	0	79.50	33.50	1.50	78.2	78.0	78.10
Semesan	.1	1	83.4	30	16	75.0	15	10	79.20	22.50	13.00	77.8	79.0	78.40
Untreated, inoc.				60	5	79.7	25	5	79.70	42.50	5.00	78.4	80.0	79.20

TABLE 4.—*Continued*

Treatment			Cusado barley									Winter Turf oat		
			Plat 1			Plat 2			Average			Germination		
				Heads in rod row			Heads in rod row			Heads in rod row				
Material	Strength of solution	Duration of soak	Germination	Covered smut	Loose smut	Germination	Covered smut	Loose smut	Germination	Covered smut	Loose smut	Plat 1	Plat 2	Average
	Pct.	Hour	Pct.			Pct.			Pct.			Pct.	Pct.	Pct.
Corona No. 620	.2	1	76.4	3	0	70.0	0	0	73.20	1.50	0	76.0	74.0	75.00
Corona No. 620	.2	½	73.2	0	0	68.0	0	0	70.60	0	0	74.8	78.0	76.40
Corona No. 620	.2	¼	80.4	0	0	76.0	0	0	78.20	0	0	73.6	70.0	71.80
Corona No. 620	.1	1	88.4	1	0	70.0	1	1	79.20	1	.50	75.4	75.0	75.20
Corona No. 620	.1	¼	88.0	0	0	72.0	0	1	80.00	0	.50	76.4	81.0	78.70
Corona No. 620	.05	1	89.8	0	0	81.0	0	0	85.40	0	0	66.4	81.0	73.70
[illegible]ate		dust	93.4	28	13	80.0	33	1	86.70	30.50	7.00	65.0	82.0	73.50
Du[illegible] No. [illegible]		dust	86.4	9	4	83.0	7	0	84.70	8.00	2.00	75.6	87.0	81.30
Formaldehyde, 1:320		⅙	76.2	4	0	69.0	4	0	72.60	4.00	0	73.2	72.0	72.60
Untreated, inoc.				70	4		14	0		42.00	2.00	84.0	79.0	81.50
Jacobsohn's No. 1	.3	1	85.0	1	0	83.0	0	0	84.00	.50	0	80.0	74.0	77.00
Jacobsohn's No. 1	.2	1	87.4	0	0	78.0	2	0	82.70	1.00	0	83.0	79.0	81.00
Jacobsohn's No. 2	.3	1	82.8	0	0	84.0	0	0	83.40	0	0	81.0		81.00
Jacobsohn's No. 2	.2	1	90.4	0	4	73.0	6	0	81.70	3.00	2.00	81.0	85.0	83.00
Jacobsohn's No. 5	.3	1	75.8	0	0	74.0	1	0	74.90	.50	0	85.0	76.0	80.50
Jacobsohn's No. 5	.2	1	85.8	0	0	81.0	2	0	83.40	1.00	0	84.0	81.0	82.50
Corona No. 10	.2	1	88.0	0	0	67.0	0	0	77.50	0	0	92.0	80.0	86.00
Dupont S.D. No. 11-1	.25	1	90.4	0	0	76.0	0	0	83.20	0	0	85.0	75.0	80.00
Untreated, inoc.				20	0		35	0		27.50		86.0	76.0	81.00

are averages of duplicate plats, while those for 1924 are averages of three replications. Some of these materials were not used in all three years, because (1) they were not available; (2) others were discarded due to lack of effectiveness; and (3) still others were liquids which were not considered so satisfactory as dusts for the control of bunt and therefore were discarded. A larger number of the new compounds were used in rod-row tests, but no bunt occurred even in the controls, so these data were not tabulated.

All factors considered, copper carbonate proved to be the most satisfactory of all the treatments listed in table 5, although it was hardly as effective as copper-sulphate-lime in preventing bunt. In the final averages, Chlorophol seems to have been better both from the standpoint of bunt control and yield. The difference in average yield is due to the fact that Chlorophol was not used in the 1924 crop when the yields were low. Copper carbonate proved better than Chlorophol in both years when the latter was used. Coppercarb, which has a much lower percentage of metallic copper than is contained in the pure copper carbonate, produced excellent results in 1924.

Oat Smuts. Only a few materials were used on oats sown in fortieth-acre plats in 1921 and 1923. In the fall of 1921 only a few were available, and, at the time of fall sowing in 1923, many had been discarded. A larger number of compounds were used on seed sown in 1922, but unfortunately very little smut developed in the controls, so the experiments were worthless from the standpoint of determining smut control. At the same time, the stands were so irregular, because of winter killing and non-uniform soil, that the yield records at harvest time in 1923 were worthless. Again, in 1924, little smut developed, even though the seed was scarified before inoculating, and therefore only the yield records are given. During these two years, however, most of the plats produced a trace of smut, but not so much as occurred in the control plats. Table 6 gives the records taken from duplicate or triplicate plats for each treatment. In the fall of 1921 the seed was divided into two parts; one part was inoculated with loose smut, and the other with covered smut. Duplicate plats from seed of each treatment of each of these two seed lots were sown. In the fall of 1923 the two smuts were mixed and all the seed was inoculated with the mixture, and triplicate plats from seed of each treatment were sown.

The results in table 6 show that Chlorophol had a beneficial effect on germination and yield and at the same time controlled both smuts in 1922, the only year when smut appeared. Copper carbonate dust was beneficial to germination and yield but failed to give satisfactory smut control. Some others of the mercury materials have shown good effects on germination and

TABLE 5.—*Effects of certain disinfectants on emergence, bunt infection, and yields of Purplestraw wheat (C.I. 1915) grown from treated seed on fortieth acre plats on Arlington Farm, Virginia, in the three years 1922–1924, inclusive*

Treatment			Average emergence, bunt infection, and yield											
			1922			1923			1924			2 or 3 year[b]		
			Per cent			Per cent			Per cent			Per cent		
Material	Strength of application	Duration of soak	emergence	bunt	Bushel per acre	emergence	bunt	Bushel per acre	emergence	bunt	Bushel per acre	emergence	bunt	Bushel per acre
	Pct.	Hour												
Untreated, inoc.			76.25	52.17	21.8	57.0	0.89	29.9	76.33	78.6	5.4	69.86	43.89	19.0
Copper carbonate (C.P.)	2 oz. bu.	dust	77.75	0.80	33.0	67.0	0.0	36.4	77.0	0.0	18.4	73.92	.27	29.3
Chlorophol	.3	1	80.00	0.38	31.1	65.0	0.0	34.5				72.50	.19	32.8
Formaldehyde 1:320		1/6	76.75	0.37	30.7									
Copper sulphate	1–10	dip	75.5	0.03	32.1	58.0	0.0	35.6	81.00	trace	15.8	71.50	.01	27.8
Lime	1–10	dip												
Seed O-San	1 oz. bu.	dust				65.0	0.0	32.9	77.00	22.0	15.6	71.00	11.00	24.2
Corona No. 620	.1	1				60.5	0.0	33.6						
Corona No. 408	2 oz. bu.	dust				72.0	0.0	36.3	82.0	2.0	20.6	77.00	1.00	28.4
Coppercarb	2 oz. bu.	dust							78.0	0.0	20.0			
Semesan	2 oz. bu.	dust							82.0	trace	18.7			
Dupont D.D. No. 8	2 oz. bu.	dust							74.0	2.0	15.3			
Dupont D.D. No. 9	2 oz. bu.	dust							80.0	1.0	16.0			

[b] Two and three-year averages should not be compared. They are included for convenience in reading the results.

TABLE 6.—*Annual and average effects of various treatments on emergence, smut infection, and yields of Winter Turf oat grown from inoculated, treated seed sown on Arlington Farm Virginia, on fortieth-acre plats in the fall of 1921 and 1923*

Treatment			Crop of 1922 inoculated with						Crop of 1924 inoculated with both smuts		
			Loose smut			Covered smut					
Material	Strength of solution	Duration of soak	Emergence	Infection	Acre yield	Emergence	Infection	Acre yield	Emergence	Acre yield	2-year average acre yield
	Pct.	Hours	Pct.	Pct.	Bu.	Pct.	Pct.	Bu.	Pct.	Bu.	Bu.
Untreated, inoc.			83.00	2.55	45.7	83.25	8.40	39.3	65.0	37.3	39.9
Chlorophol	.3	1	89.75	0.03	54.0	89.75	0.02	42.4	72.0	42.5	45.3
Formaldehyde 1:320		1/6	86.00	0.15	48.6	82.5	0.04	44.1	58.0	34.6	40.5
Formaldehyde 1:320		1/2 wash in water									
Copper carbonate		dust	88.75	0.27	50.2	82.5	1.45	37.8	64.0	30.6	
Semesan	.3	1							66.0	40.4	
Corona No. 620	.2	1							60.0	42.0	
Furfural (C.P.)	.5	1							32.0	16.5	
Furfural (Com.)	.5	1							35.0	22.1	

yield, but unfortunately the results were valueless in 1923 when the largest number of them were used. Furfural proved very injurious to the seed in the strength of solution used. This strength, even though injurious to barley seed, failed to control the barley smuts. (See table 7 and plate 3–B.) No smut occurred in the oats, so that no record was obtained of its effectiveness in the control of oat smuts.

Barley Smuts. Three years' records are available on the control of barley smuts. The data for the first year, however, have been published in PHYTOPATHOLOGY (11). In the 1924 crop the percentages of smut infection were rather high in the controls, and the smut control records of the treatments therefore are rather significant. No yield data are available, however, due to winter-killing and poor development of the plants in spots throughout the plats. The seed for the 1924 crop was run through a scarifying machine before inoculating in order to break the hulls with the hope that higher percentages of smut might occur, such as were obtained by Tisdale (12) when the hulls were carefully removed with a scalpel. Rather high percentages of smut infection were obtained. This may have been due to the scarifying or to natural infestation, as heavily infested seed was used. Tennessee Winter barley was used for these experiments. The results of these tests are given in table 7.

The previously published records for 1922 are not given in this table. Chlorophol was the only organic mercury compound used in these 1922 experiments. Seed of both Han River and Tennessee Winter barleys was treated. The results (11) show that, from the standpoint both of smut control and of yield, Chlorophol was superior to formaldehyde, hot water, and copper carbonate. Copper carbonate failed to control the smuts of barley.

The figures in table 7 show that several of the organic mercury compounds were superior to formaldehyde from the standpoint of seed germination, smut control (Plates 2, 3, and 4), and yield of plants from treated seed. The highest yields were obtained with Semesan .3 per cent (1-hour soak), Chlorophol (Plate 4–A) .3 per cent (1-hour soak), and Corona No. 620, a .2 per cent solution (¼-hour soak). From the results obtained with a .2 per cent Semesan solution, the indications are that a solution weaker than .3 per cent could not be used successfully. Corona No. 620, however, apparently can be used in very weak solutions with good results (Plate 2–A). This would be of considerable advantage in the use of such expensive materials as the mercury compounds. Formaldehyde followed by a water bath was not as effective as formaldehyde alone (Plate 2–B). The dusts failed to control smuts of barley (Plate 3–A). Furfural did not control smut and injured the seed (Plate 3–B). In fact, the percentages of smut were higher than in the controls (Plate 4–B).

TABLE 7.—*Effects of seed treatments on emergence, smut control, and yield of Tennessee Winter barley sown in duplicate for 1923 and triplicate for 1924 in fortieth-acre plats on Arlington Farm, Rosslyn, Virginia. Crops of 1923 and 1924*

Treatment			Average emergence, infection, and yield									
			Crop of 1923				Crop of 1924			Two-year		
Material	Strength of solution	Duration of soak	Emergence	Covered smut	Loose smut	Per acre	Emergence	Covered smut	Loose smut	Emergence	Covered smut	Loose smut
	Pct.	Hours	Pct.	Pct.	Pct.	Bu.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
Untreated, inoc.			77.9	14.2	5.1	33.8	76.0	25.31	4.19	76.95	19.75	4.64
Dupont Dust Disinfectant No. 12		dust	84.7	7.6	1.7	33.6						
Chlorophol	.3	1	81.4	trace	trace	45.5	69.0	trace	trace	75.20	trace	trace
Uspulun	.3	1	82.8	trace	trace	38.3				82.80	trace	trace
Germisan	.3	1	82.7	trace	trace	44.7				82.70	trace	trace
Semesan	.3	1	81.3	trace	trace	47.6	78.0	trace	0	79.65	trace	0
Semesan	.2	1	79.5	18.5	2.2	41.9				79.50	18.50	2.20
Formaldehyde 1:320		1/6	72.6	17.1	0.3	35.4	70.0	4.93	trace	71.30	11.01	0.15
Formaldehyde 1:320		1/2 wash in water					74.0	23.81	1.19			
Copper carbonate		dust	86.7	15.8	3.1	33.1						
Untreated, inoc.			77.9	21.4	2.2	39.5	76.0	27.62	2.62	76.95	24.51	2.41
Corona No. 620	.2	1	73.2	trace	trace	41.5	76.0	trace	trace	74.60	trace	trace
Corona No. 620	.2	1/2	70.6	trace	trace	39.7						
Corona No. 620	.2	1/4	78.2	trace	trace	45.2						
Corona No. 620	.1	1	79.2	trace	trace	43.3	76.0	4.0	trace	77.60	2.0	trace
Corona No. 620	.05	1	85.4	trace	trace	43.1						
Furfural (C.P)	.5	1					not taken	31.2	4.27			
Furfural (Com.)	.5	1					68.0	30.0	3.73			
Corona No. 408		dust					80.0	11.33	4.87			
Untreated, inoc.			77.9	14.5	2.25	40.3	76.0	26.80	2.80	76.95	20.65	2.52

In the fall of 1923 several varieties of barley were sown in fortieth-acre plats after treating with Semesan, one of the chlorphenol-mercury-sulphate compounds. Adjacent to each treated plat was one sown with comparable untreated seed of the same variety. The striking results of these treatments, based upon the averages of duplicate plats, are given in table 8. Semesan was used for these experiments because a supply was on hand at the time. Other organic mercury compounds might have been used with good results.

TABLE 8.—*Effects of Semesan (.3 per cent solution, 1 hour soak) on the control of smut and the yields of winter barleys, grown in duplicate fortieth-acre plats on Arlington Farm, Rosslyn, Virginia, crop of 1924*

Plat No.	Variety	Treatment	Average infection and yield		
			Covered smut	Loose smut	Per acre
			Pct.	Pct.	Bu.
1 & 36	Wisconsin Winter (C.I. 2159)	Semesan	0	0	40.75
2 & 37	Wisconsin Winter (C.I. 2159)	Untreated	21.7	1.25	31.10
9 & 44	Orel (C.I. 351)	Semesan	0	0	43.10
10 & 45	Orel (C.I. 351)	Untreated	trace	trace	39.2
22 & 57	Tennessee Winter (Sel. 52)	Semesan	0	0	45.3
23 & 58	Tennessee Winter (Sel. 52)	Untreated	32.7	5.35	37.65

The data in table 8 show that Semesan was very effective in the control of the barley smuts in these varieties. The yields were much better from plats grown from treated seed. There was considerable improvement in the yield of Orel from treated seed even though there was only a trace of either smut in plants from untreated seed. No careful records were made of the occurrence of other diseases which might have been controlled. The plants from Semesan-treated seed outyielded the neighboring plants from untreated seed in all six instances. Barley seedlings from seed treated with Semesan and sown on Arlington Farm in the fall of 1924 also are showing the beneficial effects previously mentioned (Plate 1). The seed was taken from uniform lots of machine-threshed seed grown on Arlington Farm in the previous season.

DISCUSSION

In preliminary experiments a number of new seed disinfectants produced a beneficial effect on germination of the treated seed and at the same time gave promise of good smut control. Among these materials copper carbonate dust, as reported by other investigators, produced excellent control of bunt. The stands of grain from treated seed and the yields also were improved. It failed, however, to satisfactorily control the smuts of oats and barley. Among the more promising of the mercury compounds used in these preliminary tests were Chlorophol, Corona No. 620, Germisan, Semesan, and Uspulun.

The more promising materials were used in experiments in fortieth-acre plats from which yield data were obtained. In these fortieth-acre experiments copper carbonate proved the most satisfactory material for the control of bunt in wheat. The germination of the seed was improved, bunt was satisfactorily controlled, and the yields were good as compared with those of other treatments. The copper carbonate treatment is less expensive than those with the mercury dusts now being prepared. Some of the liquid-mercury treatments gave excellent results in the control of bunt, but their use is not considered practicable in view of the highly satisfactory results obtained with the cheap and easily applied copper-carbonate dust. Only dusts which are either more effective or less expensive, or both, are likely to compete successfully with copper carbonate.

Copper carbonate, however, failed to cause entirely satisfactory control of oat smuts and reduced the smuts of barley only slightly. It showed beneficial effects on germination of these grains, but the lack of control of the smuts makes it undesirable. So far, none of the dust treatments has proved entirely satisfactory for the control of the smuts of oats and barley. For this reason the liquid treatments were used more extensively on the seed of these crops. The failure to obtain smut infection in the untreated plats of oats in two of the years leaves insufficient data to warrant any definite conclusions concerning the fungicidal value of these new materials in controlling smuts of fall-sown oats. The writers believe, however, that liquid treatments are necessary for best results, and that formaldehyde should be recommended until we have positive proof of the superiority of some other material.

In the control of barley smuts, several of the organic mercury compounds, including Chlorophol, Corona No. 620, Germisan, Semesan, and Uspulun have given excellent results. In the experiments herein described the results with these compounds undoubtedly are superior to those obtained with formaldehyde from the standpoint of seed germination, smut control, and yields of plants from treated seed. As has been previously reported, both smuts have been controlled satisfactorily. These materials, however, are more expensive than formaldehyde and also are poisonous. The additional expense, however, is more than compensated by the increased germination and yields. There also is a possibility of saving seed by sowing less of the treated seed. Care should be used with treated grain to prevent poisoning of animals.

According to Henning (5), and others, the active ingredients in the mercury compounds are absorbed rapidly by the seed and the solutions become weaker with use. This would render them less desirable. However, this could be overcome by adding a new supply of the material after each lot of seed is removed from the solution. The quantity to be added would have to be determined on the basis of strength desired and the volume of solution in question.

Formaldehyde, followed by a wash in clear water, as recommended by Henning, does not cause the seed injury often resulting from formaldehyde alone, but in the case of barley the control of smuts was not so satisfactory as was the control with formaldehyde alone.

Some of these new disinfectants have had a beneficial effect in most cases on the germination of machine-threshed seed taken from uniform seed lots of pure varieties. The yields have been improved in many cases. In some cases the increase in yield has been more than could be accounted for through smut control. In table 8 it will be noted that where only a trace of smut occurred in Orel barley, plants from seed treated with Semesan yielded better than plants from untreated seed. This difference, however, was not great. It is possible that it could have been caused by other organisms on the seed or in the soil which injured germination and subsequent development of seedlings from untreated seed.

Excluding the contamination from smut, the seed sown in these experiments probably was as good as or better than the average lot of seed sown by the farmer. When the percentages of germination were high, the improvement by treatment with these compounds was less pronounced. This would seem to indicate that if it were possible to eliminate all parasitic and probably certain saprophytic organisms, both on the seed and in the soil, which attack the germinating seed, little benefit would be derived by treating seed of high vitality. In other words, there is a question as to whether these treatments actually cause stimulation. Of course, elimination of the disease factors mentioned above is not possible in farm practice, but an approach to it through seed treatment is possible and worth while.

On the whole, copper carbonate dust caused the most satisfactory control of bunt in wheat. The organic mercury compounds have given promising results in the control of oat smuts but have not been superior to formaldehyde in the limited experiments conducted by the writers. For the control of barley smuts, however, they have been the most satisfactory fungicides.

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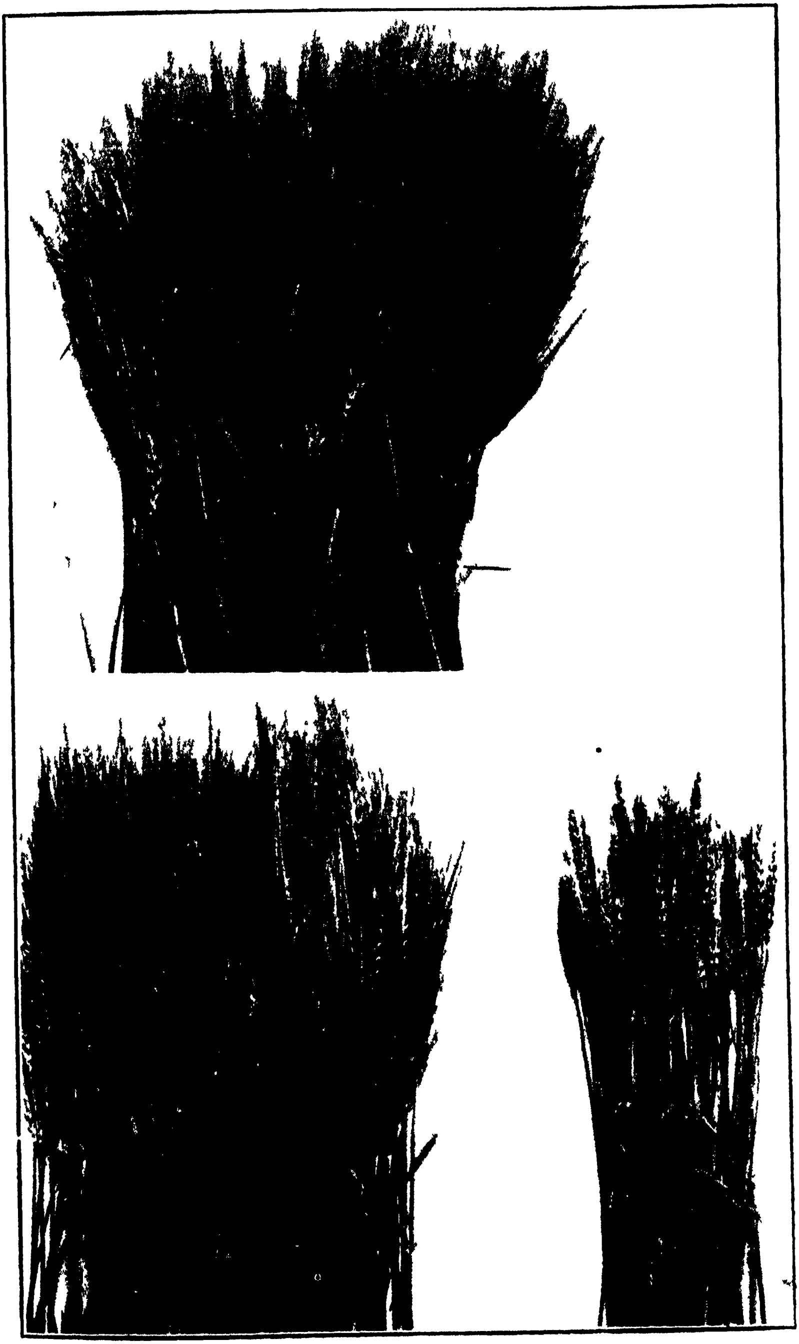
PLATE XXVIII



A PYRENOMYCETOUS LEAF SPOT OF BUR CLOVER

L. E. MILES

WITH FOUR FIGURES IN THE TEXT AND PLATES XXIX AND XXX

On bur clover near Auburn, Alabama, there occurs very commonly a leaf-spot characterized by minute dark lesions dotted over the surfaces of the leaflets, stipules, and petioles. My attention was first called to this leaf-spot by Dr. E. F. Hopkins, who in 1920 made some preliminary notes and investigations regarding it. Dr. Hopkins has given me his notes, and I wish to take this opportunity to make acknowledgment to him of the great help which his sketches and suggestions have been to me.

The disease first appears in the early spring. Investigation has shown that the causal fungus is a species of *Pleosphaerulina*, similar to that on alfalfa, *Pleosphaerulina briosiana* Pol., and not greatly dissimilar from that on clover described by Dr. Hopkins from the campus of the University of Missouri, *Sphaerulina trifolii* Rostr. Evidence will be presented later which will show that it is different from either of these, and since no reference to it is to be found in the literature, the author has described it as a new species.

Although the disease is very common near Auburn, it is not conspicuous. It has not been encountered by the author in other localities. Many leaves are yellowed and killed by it, but its ravages are so greatly overshadowed by another leaf-spotting fungus, *Cercospora medicaginis*, that it very readily can be overlooked. The loss caused by it is probably not great, although it may cause considerable defoliation under favorable conditions.

SYMPTOMS

The first signs of the disease are minute black specks on the leaf-blades, stipules, and petioles. These lesions are very small and when few in number do not appear to injure the leaf, but when they are numerous the leaf tissue between the spots turns pale green to yellow. The spots then stand out in much greater contrast to the surrounding tissues, and the leaf has the appearance of being peppered. The spots do not enlarge very greatly, although occasionally they may reach a diameter of about one millimeter. Both the upper and lower sides of the leaves bear lesions, although they are usually more abundant on the lower surfaces and on the stipules and petioles. When the petioles become severely infected, the leaflets, even though they themselves may bear no lesions, turn pale yellow, and fall. The presence of numerous infections on the leaflets, with or without petiole lesions, results in similar discoloration and defoliation. The contrast between these lesions and the surrounding pale yellow leaf tissue is very strong. Young lesions, even when few in number, are very clearly distinguished when the leaf is held between the eye and the light. Such young lesions are shown in Plate 1, Fig. 1, photographed by transmitted light. Later they enlarge somewhat and become easily visible to the unaided eye, without the use of transmitted light (Plate 1, Fig. 2). A small, depressed, indefinitely delimited, gray to brown center is sometimes observed, although more commonly it is entirely absent. Under a hand lens the perithecia stand out clearly as small bright objects in which the ostiola can readily be distinguished.

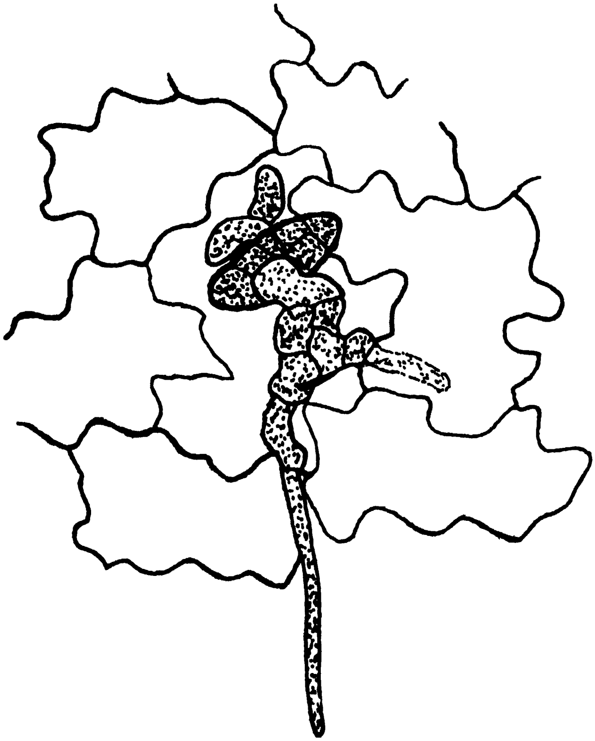


FIG. 1. Germinating ascospore on surface of leaf.

Lesions have also been observed on the stems, peduncles, calyx, corolla, and even on the seed. The latter is very significant, as it indicates a very important possible means of transmission of the pathogene. Lesions on the seed look like small sclerotia, but they possess the structure of perithecia, although no asci or ascospores have ever been found in them. That they are a means of transmitting the disease is clearly evident from the fact that viable cultures have frequently been obtained from them. Such seed lesions are shown in Plate 2, Fig. 2.

MORPHOLOGY

The mycelium of this fungus, as found naturally in infected tissue, is generally rather large in diameter, densely granular in content, and almost invariably swollen at frequent intervals. In culture on most of the agars used in this experiment it is usually much the same but more uniform. The perithecia in nature are spherical or slightly flattened on the vertical axis and have proportionately large ostiola. The perithecial walls are smoky-brown in color, thin, membranaceous, and so transparent that the asci, and even the ascospores, can sometimes be distinguished through them when thoroughly mature. The perithecia are not beaked, the ostiola being

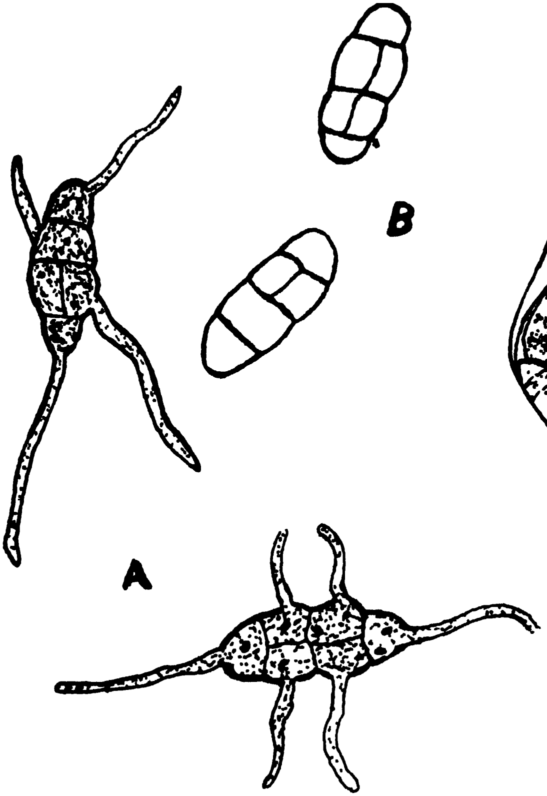


FIG. 2. (A) Germinating ascospores; (B) Ascospores; (C) Ascus containing spores

merely relatively large, approximately circular openings in the upper surface.

Young asci are pyriform in outline, becoming more or less broadly ovate with age (Fig. 2, C). In the younger stages the wall of the upper part is much thickened, gradually becoming thinner, but remaining proportionately quite thick until time for spore dispersal. There is some evidence of the presence of a pore, but usually it is not conspicuous. The asci are quite similar to those pictured by Hopkins for *Sphaerulina trifolii,* except that his are reversed, the thick portion being pictured as at the base. I am of the opinion that he is mistaken in this matter, and that in *S. trifolii* also the thickened portion of the ascus occurs at the top.

The ascospores are muriform, hyaline when young, but becoming honey-yellow at the time of, or shortly after, expulsion from the ascus. They are irregularly or subdistichously arranged. There are usually four cross walls, but it is quite common to find spores with only three (Fig. 2, B). Invariably one, and more commonly two, of the resulting spore segments are divided by a longitudinal septation. The spores are more or less symmetrical in outline, although the segments containing the muriform septation are inclined to bulge. Where four cross septations occur, the two halves of the spores are usually separated by a constriction slightly deeper and more conspicuous than elsewhere in the spore. The longitudinal septations usually occur in the middle segments, although both may occur on one side of the middle constriction; or, on the other hand, both may occur in the subterminal segments, having the central one or ones undivided. The mature spores are filled with granular protoplasmic contents and often contain several large oil drops.

ISOLATION

A considerable number of isolations have been made from leaf blades, petioles, stipules, peduncles, and seeds; and the same characteristic growth has been obtained in all cases. Growth from single ascospores has invariably been identical with that obtained from these isolations. Cultures from all sources were capable of infecting healthy leaves of bur clover Owing to the fact that the Cercospora leaf spot occurs so generally on the same host plant, cultures of *Cercospora medicaginis* E. and E. are commonly obtained together with the Pyrenomycete. Mature perithecia are not obtained under ordinary conditions of growth, but have been obtained under certain special conditions, as will be detailed later.

The fungus grew best on potato, and on cornmeal-glucose agars. It grew almost as well on prune agar. The growth is never rapid and is at first composed entirely of white mycelium. After a few days the center of the colony begins to turn dark, owing to the formation of numerous black bodies which are of the nature of immature or aborted perithecia. These bodies are filled with granular contents or occasionally with a substance resembling oil globules, which oozes out when they are crushed. The mycelium of the inner portion of the colony, at the time of formation of these bodies, takes on a golden brown to brown color, shading gradually to hyaline at the border. On prune agar, and less conspicuously so on cornmeal agar, the mycelium near the edge of the colony has a green tint. On potato agar the inner portion of the colony bearing the sclerotia or aborted perithecia possesses a silvery luster, due to the excretion of minute drops of water. In appearance the colonies differ from those of *S. trifolii* in the presence of a much more evident and much wider sterile border, and in the smaller size of the sclerotial bodies.

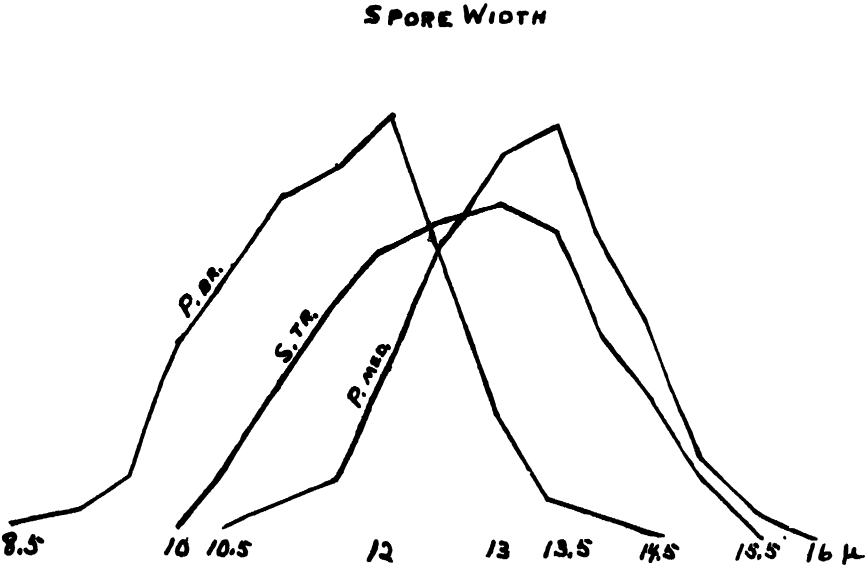


FIG. 3. Graph showing spore width variation in 200 ascospores of *Pseudoplea briosiana, Sphaerulina trifolii* and *Pseudoplea medicaginis.*

In two instances, cultures on potato agar, maintained at a temperature of from 7.5° to 10° C. for 22 days, matured spores, thereby showing the true character of the above-mentioned sclerotial bodies. The same experiment was repeated, but this time no spores matured. Some sclerotial structures, however, contained ovate, hyaline bodies which undoubtedly were immature asci. If they had been maintained for a longer time under the same conditions, there is little doubt that mature asci and spores would again have been obtained. Jones (2) has reported similar results with *Pleosphaerulina briosiana* on alfalfa.

The fungus grew best at a temperature of about 20° C. No growth occurred at 35° C. as an upper limit. The lower limit of growth was not determined. It is somewhat peculiar that the only evidences of saltation appeared at the optimum temperature for development. On two different occasions wedge-shaped saltants appeared, two developing in one colony and one in another. These saltants were characterized by a browner color of the mycelium, the brown color extending entirely to the outer edge of sterile margin where color is usually entirely absent. The perithecial bodies were smaller in these saltation areas.

PATHOGENICITY

It was extremely difficult to obtain satisfactory infections from pure cultures of this fungus, owing to the fact that no spores were produced under ordinary conditions of growth. Fragments of mycelium and sclerotial bodies usually dried out before the pathogene could become established. However, in a number of instances, with careful manipulation, infections were secured in this manner, and typical lesions were produced. Re-isolation was successfully accomplished, thereby establishing the pathogenicity of the organisms.

Table 1 presents the results of inoculations of southern bur clover (*Medicago maculata*) with cultures secured both from seed and leaf tissue It may be seen from Plate 2, Fig. 3, that these cultures are identical.

TABLE 1.—*Results of inoculation of southern bur clover with Pseudoplea from bur clover, observed 42 hours after inoculation*

Culture	Leaflets inoculated	Leaflets infected	Remarks
S[a] III	6	6	Grayish spots watersoaked, 0.4 cm.
S I	5	0	
A[b] III	5	5	Spots gray, irregular, watersoaked
A IV	7	6	
A I	4	3	
A II	5	4	
S II	9	9	One infection slight

[a] S = cultures isolated from seed.

[b] A = cultures isolated from leaf tissue.

In the main series of inoculations, however, it was found much more practical to use as a source of inoculum a quantity of leaves bearing large numbers of perithecia, suspended from the top of a bell jar which was inverted over a pot containing an uninfected plant. As a control, another pot of plants was covered with a bell jar in which no such source of inoculum was present. In all cases both bell jars were removed at the end of 42 hours.

Table 2 shows the results of inoculations in which 19 species and varieties of plants belonging to the genera *Medicago* and *Trifolium* were used. The numbers in the second column indicate approximately the relative amount of infection on the basis of 10, which represents the most severely infected.

TABLE 2.—*Results of inoculation of 19 species of Medicago and Trifolium with Pseudoplea from bur clover*

Host	Relative infection	Remarks
Medicago maculata	10	Spots very abundant, prominent on all parts of the plant except the stem.
Medicago hispida sardoa	10	Much as on *M. maculata.*
Medicago hispida compacta	8	Spots abundant, slightly less prominent.
Medicago hispida reticulata	8	As above.
Medicago hispida nigra	7	Spots numerous, prominent.
Medicago hispida confinis	6	Spots scattered, prominent, leaves only.
Medicago sativa	2	Slight watersoaked spots, developing no further.
Grimm alfalfa	2	As above.
Trifolium repens	2	Spots few, small, watersoaked.
Trifolium pratense (Red)	2	" " " "
Trifolium pratense (Mammoth)	2	" " " "
Trifolium hybridum	1	Mere watersoaked specks.
Trifolium reflexum	0	No trace of infection.
Trifolium incarnatum	0	" " " "
Trifolium procumbens	0	" " " "
Medicago ruthenica	0	" " " "
Peruvian alfalfa	0	" " " "
Turkestan alfalfa	0	" " " "
Argentine alfalfa	0	" " " "

In this experiment (Table 2) the bell jars were all removed at the end of 48 hours. At this time minute lesions were becoming evident on the leaves of most of the bur clover plants, while none of the control plants or those belonging to the genus *Trifolium* showed any traces of infection. After the bell jars were removed, the lesions on the plants of *Medicago maculata* and on all varieties of *Medicago hispida* continued to develop in size, while those on alfalfa and the clovers never attained more than a slight watersoaking or specking at the most.

It becomes evident from table 2 that southern bur clover is the most susceptible of the plants inoculated, and *Medicago hispida sardoa* only slightly less so. All other varieties of *Medicago hispida* are slightly less susceptible, but all are susceptible in the presence of abundant infection. It seems probable that none of the alfalfas or clovers are natural hosts of this organism, since the spots, when they appeared at all, did not develop further than the pin-prick stage.

SEASONAL HISTORY

Infected plants were observed occurring naturally in the open in February, 1923; as early as January 4, 1924; and on January 24, 1925. On looking for the source of inoculum, it was found that numbers of old dead leaves around the base of infected plants were covered with numerous perithecia containing asci and mature ascospores. There seems to be little reason for doubting that much of the early infection on the young plants comes from such perithecia developed on the plants of the previous season. The minute spots on these plants, as mentioned previously, gradually enlarge and produce perithecia, thereby providing more inoculum, which produces infection throughout the season. No conidial stage has ever been observed which gave any indication of being connected with the fungus in question.

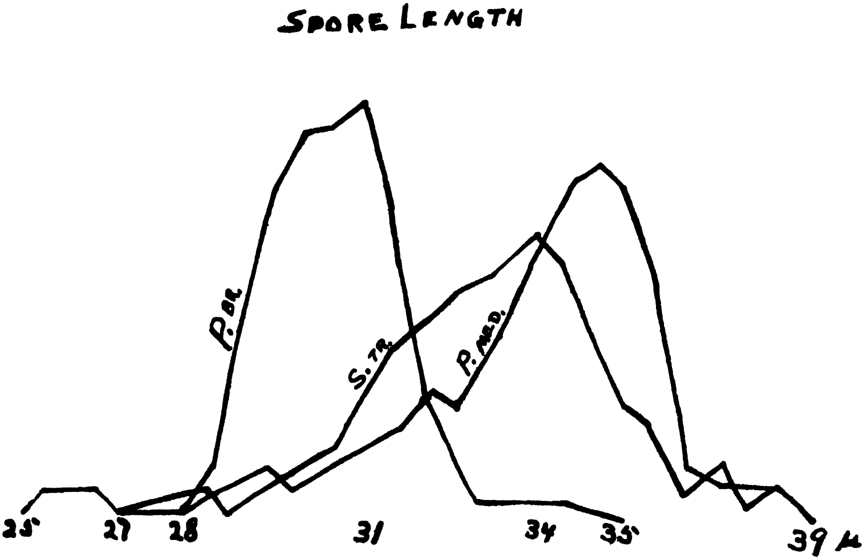


FIG. 4. Graph showing spore length variation in 200 ascospores of *Pseudoplea briosiana*, *Sphaerulina trifolii*, and *Pseudoplea medicaginis*.

As mentioned previously, small sclerotia-like bodies, having more or less the structure of perithecia, but without asci or spores, were frequently observed on the seeds. These structures were identical in character with the bodies so abundantly produced in culture under ordinary temperature conditions, and which were proved to be immature or aborted perithecia, capable of developing normal asci and ascospores under favorable conditions. It was proved repeatedly that these bodies were capable of transmitting the disease, as viable cultures were produced from them, which were identical in growth characteristics and pathogenicity with cultures isolated from other parts of the plant. Such seed have not been germinated to see if they would produce infected plants, but there would seem to be little doubt that they would do so. It is probable that they may serve as a capable and prolific agent in the spread of this disease in the field and from one locality to another.

PATHOLOGICAL RELATIONS

On coming in contact with the leaf surface, the ascospores germinate and send tubes directly through the epidermis. Any one or all of the cells of the spore may produce germ tubes (Fig. 2, A). These tubes may grow through the epidermis immediately or may wander over the surface for some distance before penetrating (Fig. 1). Sometimes the fungus produces a somewhat extensive layer of pseudoparenchymatous cells on the leaf before penetration. These are usually of a dark brown color, as viewed under the microscope, and are no doubt partly responsible for the dark color of the lesions. However, the palisade cells near the penetrating germ tube also take on a brown color which is also directly responsible for this appearance. The ascospore itself, at the time of expulsion, is hyaline or pale honey-yellow, but soon changes to a brown color similar to that of the resulting lesion. The empty wall persists for some time after infection has occurred, and it is by its presence that the nature and cause of many of the younger lesions are most readily determined. The color of the tissues around the lesion soon becomes so deep as to conceal effectively the details of further steps in its development. Most of the particular stages of spore germination, germ-tube penetration, and lesion development recorded above are much more readily observed after the leaves have been decolorized in acetic acid and alcohol. After such treatment, the muriform spores, or empty walls of the same, are to be observed in the center of almost every lesion.

SIMILAR LEAF-SPOTS ON CLOSELY RELATED HOSTS

The Sphaerulina leaf-spot of clover and the Pleosphaerulina leaf-spot of alfalfa very closely resemble this one on bur clover, both in macroscopic characters of the lesions and in the morphology of the causal organisms. The nomenclature of these two organisms has undergone in European literature a series of somewhat intricate and confusing changes. *Sphaerulina trifolii* was described on *Trifolium repens* in Denmark in 1899 by E.

Rostrup; *Pleosphaerulina briosiana* on *Medicago sativa* and *M. falcata* in Italy by Pollacci in 1901. In 1918, Von Höhnel established a new genus, *Pseudoplea,* of the Pseudosphaeriaceae, based on this latter fungus as a type, and it became *Pseudoplea briosiana* (Poll.) V. H. In 1921 the characters of this genus were amended by Petrak, who showed that Von Höhnel was wrong and that the fungus was truly Sphaeriaceous. The two species were united under the name *Pseudoplea trifolii* (E. Rostr.) Petr. It is evident however, as will be shown later, that in this union of the two species, cultural characters and inoculation experiments were not considered. The universal absence of a longitudinal cross wall in the spores of the clover organism, and the almost invariable presence of the same in the spores of the alfalfa organism are alone enough to establish the separate identities of the two, without consideration of this later evidence.

In many examinations of spores of the clover leaf-spot organism, grown on the host plant both as the result of inoculations and of natural infections, I have never observed the presence of longitudinal walls; neither does Hopkins in his description of the fungus in America, nor Rostrop in his original description, mention such walls. Specimens from Mycotheca Germanica No. 790 show none such; nor does Petrak make a direct statement that they are present, even when he places the fungus in the genus *Pseudoplea,* which he characterized as possessing them. Therefore, since the fungus can not belong to the same species or genus as the alfalfa leaf-spot organism, the author considers it best to leave it, for the present, under the name of *Sphaerulina trifolii* Rostr.

The asci of all are very similar in form, but measurements of a considerable number in each case show that there is a considerable difference in size, those of the organism causing the leaf spot on bur clover being the larger. The measurements shown in table 4 are the author's and are reached only after the examination of a considerable number of specimens In the case of *Sphaerulina trifolii,* they agree very closely with those of Hopkins for the same fungus. The graphs (Figs. 3 and 4) show very clearly the relative spore sizes; each line, for both length and breadth, is made up of the measurements of 200 mature spores taken at random. It is seen here that the average spore size, as denoted by the apex of the curve, presents a much better basis of comparison than do the figures presenting the limits of variability. It will be seen that there is a very considerable difference in the sizes of the spores of *Pseudoplea briosiana* and the fungus on bur clover. Those of *Sphaerulina trifolii* occupy an intermediate position, but approach more nearly to the latter. The average size of the *P. briosiana* spore is 31 × 12μ; that of *S. trifolii,* 34 × 13μ; and that of the bur clover organism, 35 × 13.5μ.

Table 3 shows the results obtained by inoculating twenty-two different species and varieties of clover, seven of alfalfa, and six of bur clover with spores of *S. trifolii* Rostr. The inoculum used in these tests was in all cases composed of leaves of *Trifolium repens* which contained large numbers of perithecia with mature asci and spores. As in the inoculation tests with the similar organism on bur clover, the bell jars were all removed at the end of forty-eight hours. Examinations of table 3 will show that only a few of the clovers and none of the alfalfas or bur clovers are natural hosts

TABLE 3.—*Results obtained by inoculating various species of Trifolium and related genera with Sphaerulina trifolii Rostr.*

Host	Relative infection	Remarks
Trifolium pratense (Mammoth)	10	Very abundant, prominent.
“ “ (Red)	8	Abundant, prominent.
T. repens	8	“ “
T. reflexum	6	Much the same as on *T subterraneum.*
T. subterraneum	6	Less abundant and less prominent than on *T. repens.*
T. hybridum	5	Lesions scattered but prominent.
T. alpestre	5	More abundant but less prominent than *T hybridum.*
T. agrarium	5	Lesions scattered but prominent
T. pannonicum	5	About as on *T. agrarium.*
T. squarosum	5	About as on *T. hybridum.*
T. striatum	4	Spots few but prominent
T. fragiferum	4	Lesions scattered, not prominent
T. medium	4	Scattered, not prominent.
T. incarnatum	4	“ “ “
T. carolinianum	2	Few, minute.
T. parviflorum	2	Lesions few, minute
T. procumbens	2	More abundant but less prominent.
T. glomeratum	1	Mere trace.
T. dubium	1	Mere trace of infection. Few mere watersoaked spots, progressing no further.
T. lupinaster	1	Few mere watersoaked spots.
T. alexandrinum	0	No trace.
T. angustifolium	0	“ “
Kansas alfalfa	3	Few scattered lesions, remaining small
Grimm “	3	As on Kansas variety.
Turkestan “	2	Few, minute.
Peruvian “	1	Mere trace.
Argentine “	1	“ “
Medicago sativa	2	Lesions few, minute, no perithecia developed.
M. ruthenica	2	Same as *Medicago sativa.*
M. maculata	2	Lesions scattered, minute.
M. hispida compacte	1	Mere trace of incipient infection.
M. “ *nigra*	1	Few watersoaked specks.
M. “ *reticulata*	0	None.
M. “ *sardoa*	0	“
M. “ *confinis*	0	“

for *Sphaerulina trifolii,* as on such hosts the infections, when evident at all, never progress beyond the incipient stage.

The case of the alfalfa leaf-spotting fungus, however, is different. It appears most clearly to belong to the Sphaeriaceous genus *Pseudoplea* Von Höhnel, as amended in 1921 by Petrak, and should henceforth be called *Pseudoplea briosiana* (Poll.) V. H. The name *Sphaerulina trifolii* Rostr. should be removed from the synonymy.

Comparative measurements of the perithecia of the three organisms (Table 4) show that those of *P. briosiana* on alfalfa are very nearly identical in size with those of the bur-clover organism, but that both are larger than those of *S. trifolii.*

TABLE 4.—*Comparative measurements of perithecia, asci and spores*

	Pseudoplea briosiana (Poll.) V. H.	*Sphaerulina trifolii* Rostr.	Bur clover organism
Perithecia	100–140 µ	80–125 µ	100–150 µ
Asci	68–83 × 34–40 µ	60–72 × 34–44 µ	62–92 × 38–45 µ
Spores	25–35 × 8.5–14.5 µ	27–39 × 10–15.5 µ	28–39 × 10.5–16 µ

After careful consideration of these differences in morphology, macroscopic appearance, and pathogenicity in the fungi under consideration, it has seemed clear to the author that the bur-clover organism is distinct from either of the others, belonging to the same genus as the one causing the similar disease on alfalfa, *Pseudoplea briosiana* (Poll.) V. H., but being a distinct species. The name *Pseudoplea medicaginis* sp. nov. is therefore suggested for this organism.

Pseudoplea medicaginis sp. nov.

Spots on leaves none or pale yellow to brown, indefinite in outline and size. Minute black or dark brown lesions scattered over these lighter areas or over the normally colored leaflet give it a peppered appearance. Such lesions also occur on peduncles, petioles, calyx, corolla, and seeds. On the seed they have the appearance of minute sclerotia. Perithecia numerous, spherical or slightly flattened, with large round ostiole, 100–150µ. The walls are smoky brown in color, thin, membranous, and almost transparent. Asci are few in number, pyriform in outline, or in age broadly ovate, with walls much thickened toward the apex, 63–92 × 38–45µ. Spores are eight in number, muriform, irregularly or subdistichously arranged, with four or more, rarely three, cross walls, and one, or more commonly two, of the segments divided by a longitudinal septation. The spores are first greenish hyaline in color but at maturity become almost honey yellow. They measure 28–39 × 10.5–16μ. Habitat: On leaves, stems, peduncles, petioles, calyx, corolla, and seeds of *Medicago maculata* about Auburn, Alabama.

Pseudoplea medicaginis sp. nov.

Maculis in foliis nullis vel arescente pallescentibus, indeterminatis; peritheciis numerosis, in parte folii ochraceolutescente, sparsis vel subgregariis, membranaceis, immersis, dein erumpentibus, globoso-depressis, glabris, 100–150μ, ostiolo latiusculo impresso perforatis; ascis paucis, aparaphysatis, octosporis, crasse pyriformibus, apice valde incrassatis, 63–92 × 38–45μ; sporidiis oblongo-fusoideis, utrinque obtusiusculis, initio chlorino-hyalinis, dein pallide brunneo-flavis, sursum in ascis irregulariter distichis, deorsum monostichis, muriformibus, transverse 3–4 septatis, loculis 1–2 septis longitudinalibus divisis, 28–39 × 10.5–16μ.

Hab: In foliis, caulibus, petiolis, pedunculis, sepalis, corollis, etiam semenibus Medicaginis maculatae, Auburn, Alabama.

SUMMARY

Evidence is presented in this paper to show that a Pyrenomycetous fungus producing a leaf spot on bur clover near Auburn, Alabama, is distinct from either of those causing similar spots on alfalfa and clover respectively, although the host plants are closely related and the lesions produced are very similar macroscopically.

Sclerotioid bodies on the seed were capable of producing viable cultures on agar.

Similar bodies, produced in culture, were proved to be immature perithecia, capable of developing mature asci and ascospores under favorable conditions.

All species and varieties of bur clover inoculated were found to be susceptible to the fungus, while none of the alfalfas or clovers produced typical lesions. The fungus is described as a new species, *Pseudoplea medicaginis* sp. nov.

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EXPLANATION OF PLATES

PLATE XXIX

FIG. 1. Lesions on young leaves photographed by transmitted light.

FIG. 2. Lesions on old leaves and petioles.

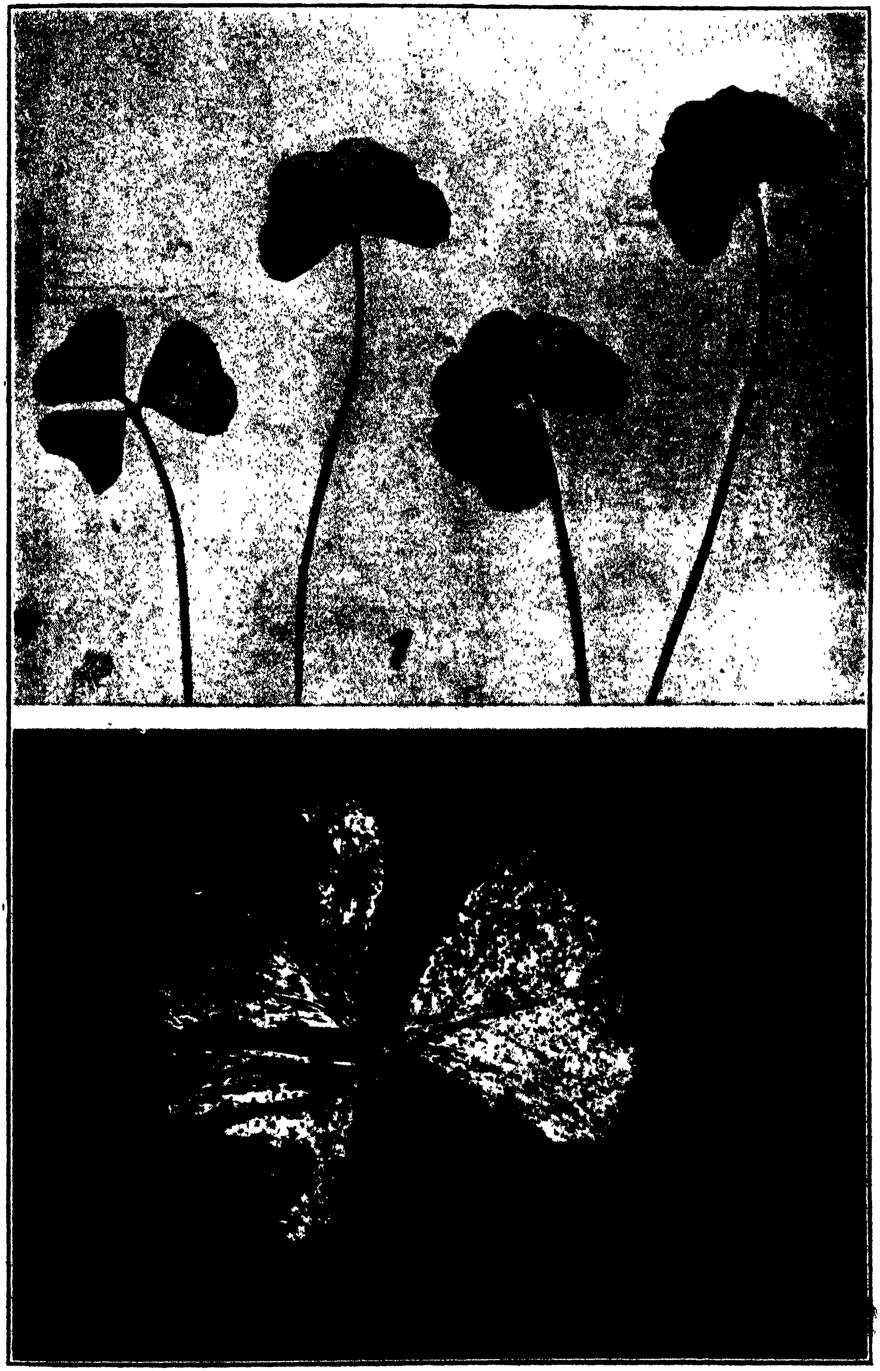
PLATE XXX

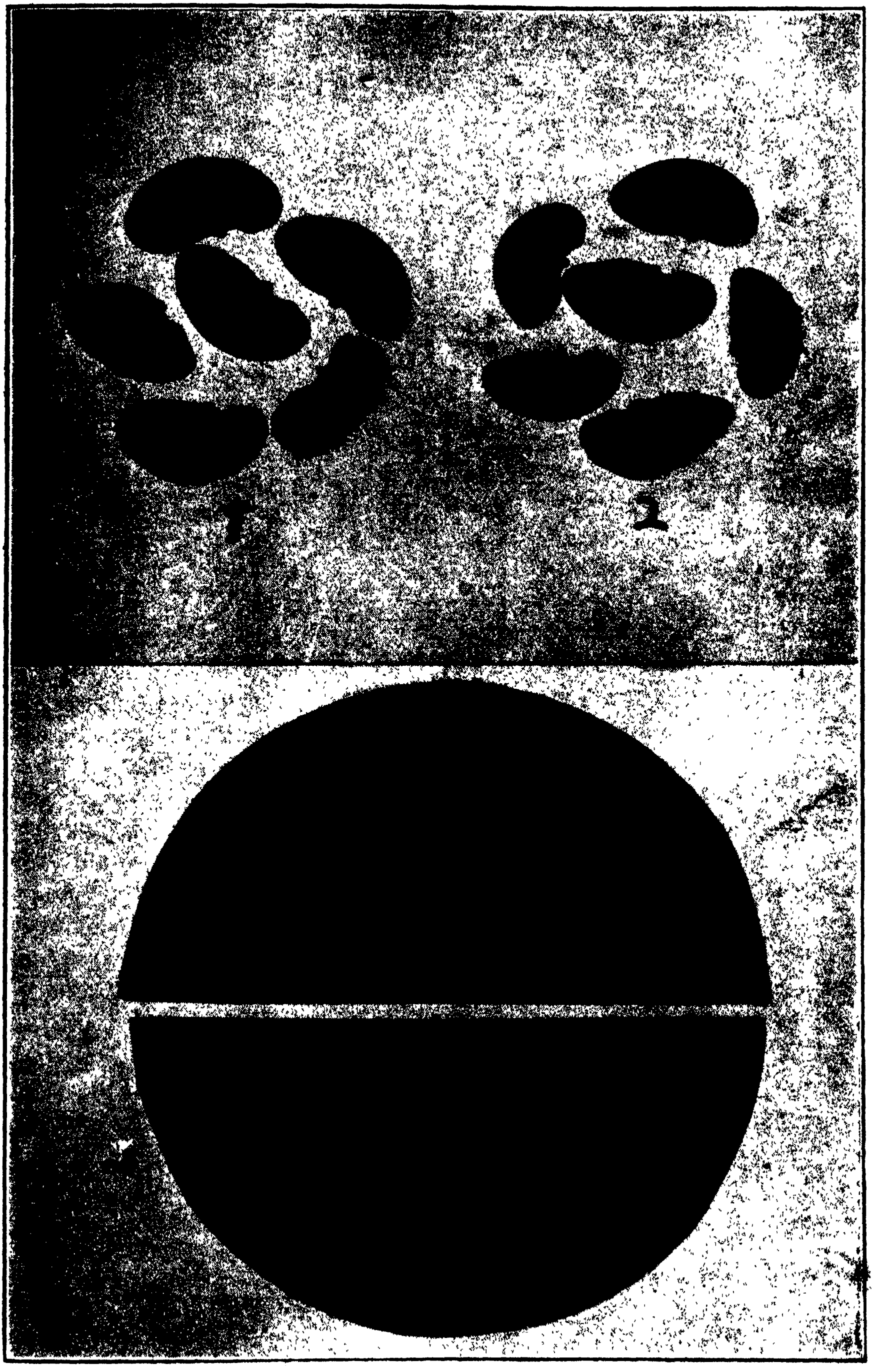
FIG. 1. Seed free from infection.

FIG. 2. Seed showing on their surface lesions having appearance of small sclerotia.

FIG. 3 (A). Culture of *Pseudoplea medicaginis* sp. nov. on cornmeal agar, isolated from leaf tissue.

FIG. 3 (B). Culture of *Pseudoplea medicaginis* sp. nov. on cornmeal agar, isolated from seed lesion.

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WEBSTER, A COMMON WHEAT RESISTANT TO BLACK STEM RUST[1]

E. C. STAKMAN, M. N. LEVINE AND FRED GRIFFEE[2]

WITH ONE FIGURE IN THE TEXT

INTRODUCTION

Webster, C. I.[3] 3780, a recently named (2, p. 66) variety of common wheat, *Triticum vulgare* Vill., introduced from Russia by the United States Department of Agriculture in 1913, apparently is resistant to more physiologic forms of *Puccinia graminis tritici* (Pers.) Eriks. and Henn. than any other common wheat yet tested in the United States. This variety seems to be so generally resistant that it is interesting and may be important. It is an awned, hard red spring variety. Although Webster wheat is not a very desirable type, it probably is potentially valuable as a parent of rust-resistant hybrids. This is particularly true because no varieties of *T. vulgare* are known to be resistant to all of the thirty-nine physiologic forms of *P. graminis tritici* thus far found in North America. For this reason, breeders have crossed many varieties of *T. durum* Desf. and *T. dicoccum* Schrk. with those of *T. vulgare,* hoping to obtain hybrids resistant to most physiologic forms. While evidently it is possible to combine the rust-resistant characters of some of the durum and emmer wheats with the botanical characters of common wheats, certain difficulties have been encountered (3). The common wheats have forty-two chromosomes (diploid), the durum and emmer varieties have twenty-eight, and einkorn has fourteen. When varieties with different chromosome numbers are crossed, many segregates are eliminated by sterility due to chromosome incompatibility (5, 8, 9, 14). Therefore there is a tendency for the progeny to revert to one or the other of the parental types. There is a high degree of sterility in crosses between durum wheat or emmer and the common wheats, and there also is some linkage between durum and emmer characters and rust resistance. This makes it necessary to use many individuals in breeding work, and it increases the difficulty of obtaining the desired results. Hence it is advantageous to have several resistant varieties of common wheat having forty-two chromosomes.

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[1] Cooperative investigations between the Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Minnesota. Published with the approval of the Director as Paper 541 of the Journal Series, Minnesota Agricultural Experiment Station.

[2] Seed of this wheat was sent to the writers by E. S. McFadden, of Webster, Day County, South Dakota, who first observed its resistance at Highmore, S. D., in 1917. A brief account of Mr. McFadden's observations has been published in The Dakota Farmer 45: 102. 1925.

[3] C. I. = Accession number of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

KNOWN RUST-RESISTANT VARIETIES OF COMMON WHEAT

It often is stated that Kanred and Kota are the only varieties of common wheat known to be resistant to black stem rust. This statement is not quite accurate. There are at least thirty-nine physiologic forms of *Puccinia graminis tritici*, but neither Kanred nor Kota, nor any other common wheat, is known to be resistant to all of them. The resistance of a variety should be considered with reference to particular physiologic forms. Marquis, generally considered to be susceptible to black stem rust, is very resistant to thirteen of the thirty-nine physiologic forms of *P. graminis tritici*. Marquis rusts commonly in the field in the upper Mississippi Valley, and in various other parts of the United States; but in some of the Gulf states, and in certain sections in the Pacific Northwest, where it is not grown commercially, it is usually resistant. Kanred is immune from eleven and resistant to seven of the thirty-nine forms, whereas Kota is immune from two and resistant to eighteen. While Kanred and Kota are the most generally resistant of the common wheats grown in the upper Mississippi Valley, some physiologic forms can infect them normally, and they sometimes are rusted heavily in the field. Hence it is important to obtain other common wheats resistant to more physiologic forms than those to which Kota, Kanred, and Marquis are resistant. Webster is resistent to all of the physiologic forms with which it has thus far been inoculated

COMPARATIVE RESISTANCE OF WEBSTER AND OTHER COMMON WHEATS

Webster has been inoculated with nineteen physiologic forms of *P. graminis tritici*, including some of the most virulent. The other forms were not available. Table 1 summarizes the reactions of the following wheats to nineteen physiologic forms: Webster, C. I. 3780; Marquis, C. I. 3641; Kanred, C. I. 5146; Kota, C. I. 5878; Mindum, C. I. 5296; and Acme, C. I. 5284. The last two varieties listed are durums which often are resistant in the field and are included for comparison.

It will be seen from table 1 that Webster is not really susceptible to any of the nineteen physiologic forms with which it was inoculated. Marquis is susceptible to fourteen of them, Kanred to eight, Kota to twelve, Mindum to thirteen, and Acme to eighteen. Arnautka, C. I. 4072, often is considered to be resistant in the field, but it is susceptible to thirteen of the nineteen forms to which Webster is resistant. Even Vernal emmer, C. I.

TABLE 1.—*Comparative reactions of Webster, Marquis, Kanred, Kota, Mindum, and Acme to nineteen physiologic forms of Puccinia graminis tritici in the greenhouse*

Physiologic form	Variety and reaction[a]					
	Webster C. I. 3780	Marquis C. I. 3641	Kanred C. I. 5146	Kota C. I. 5878	Mindum C. I. 5296	Acme C. I. 5284
1	3 –	4 –	0	3 +	1	3 ++
3	3	4 –	4 =	3 +	1 =	3 ++
9	3 =	4 –	0	3 ++	4 =	3 ++
11	3^{c}	4 =	3 ++	3 +	4 =	3 ++
15	2 – to 3 –	4 –	4 =	3 ++	4 =	3 ++
17	2 – to 3 –	4 –	0^{n}	3 +	4 =	3 ++
18	$3-^{c-}$	4 –	4 =	3 ++	1 =	3 ++
19	2 to 3	2 –	0^{f}	3 –	4 =	3 ++
21	2 to 3^{c+}	4	0	3 ++	4 –	3 ++
27	0^{f}	2	0	0^{n}	1	3 ++
29	$2\pm^{n}$ to 3^{n+}	4 –	0	3	X ±	X +
30	3 –	4	0^{n}	3 ++	X ±	X +
32	3 =	4 =	4 =	3 +	X ±	X +
33	$2+^{c}$	2	4	1 +	1 –	3 ++
34	3 =	4 –	4 -	4 =	4 =	3 ++
36	3^{n}	4	4 –	3 ++	1 =	3 ++
37	3 =	4 –	0	3 ++	4 =	3 –
38	$3=^{c+}$	2 =	4 +	3 +	X +	X +
39	2^{c+}	2 +	4	3 +	3 +	3 +

[a] Explanation of symbols: 0, immune; 1 and 2, very resistant; 3 and 3 –, moderately resistant; 3 +, moderately susceptible; 4 = to 4 +, very susceptible; X, indeterminate (probably susceptible); c, chlorosis; n, necrosis; f, hypersensitive flecks. The minus and plus signs indicate fluctuations within the type; thus, 3 = indicates a very weak type 3 infection; 3 + + indicates a very heavy type 3 infection; a ± after the X indicates that small and large pustules are about equally numerous. For further details see Stakman and Levine (11).

3686, which is almost immune from many forms, is completely susceptible to four of the nineteen.

The possible value of Webster in developing rust-resistant varieties is evident from the data given in table 2. It is resistant to five forms to which none of the other common wheats are known to be resistant. It is likely that it also is resistant to other forms. It will be seen from table 2 that no common wheat is known to be resistant to Forms 12, 13, and 20. However, the known resistant common wheats between them are resistant to thirty-six of the thirty-nine physiologic forms which the writers have isolated. The importance of this fact for breeding work is obvious.

THE TYPE OF RESISTANCE OF WEBSTER

Webster is not entirely immune from any of the physiologic forms with which it has been inoculated, although it is almost immune from the rather

TABLE 2.—*Physiologic forms of Puccinia graminis tritici, and varieties of common wheat resistant to them*

Physiologic forms and resistant common wheats							
1	2	3	4	5	6	7	8
Webster Kanred	Marquis Kanred Kota	Webster	Marquis Kanred Kota	Kanred	Marquis Kanred Kota	Marquis Kota	Kanred
9	10	11	12	13	14	15	16
Webster Kanred	Marquis Kota	Webster			Marquis Kanred Kota	Webster	Marquis Kanred Kota
17	18	19	20	21	22	23	24
Webster Kanred	Webster	Webster Marquis Kanred		Webster Kanred	Kota	Marquis Kanred Kota	Kanred Kota
25	26	27	28	29	30	31	32
Kota	Kanred Kota	Webster Marquis Kanred Kota	Marquis Kanred Kota	Webster Kanred	Webster Kanred	Kota	Webster
33	34	35	36	37	38	39	
Webster Marquis Kota	Webster Marquis Kota	Kota	Webster	Webster Kanred	Webster Marquis	Webster Marquis	

weakly parasitic Form 27. The type of infection of the other forms ranges from 2 to 3. It will be noted from table 1 that many of the forms cause a type 3 infection, but a variety which consistently develops a type 3 infection in the greenhouse is not likely to be injured much in the field. The type 3 was placed in the susceptible class by Stakman and Levine (11). From the standpoint of parasitism, the writers still think this is correct, because the genetic factors for the development of the type 3 infection seem to be more closely related to those for a type 4 than to those for types 2 and 1. But the type 3 could equally well be placed in a moderately resistant class.

Many uredinia may develop on Webster, but they are always small and seldom coalesce. Often they are surrounded by chlorotic or even necrotic areas, which is an additional indication of resistance. While this variety undoubtedly will rust in the field, it is unlikely that it will be injured severely unless it is susceptible to some of the forms to which its reaction is not yet known. The type of reaction seems to be somewhat similar to that of White Tartar oats, C. I. 551. White Tartar is not immune from the physiologic forms of *P. graminis avenae* Erikss. and Henn., which are known to occur in the United States, but it is resistant to them. They cause a type 3 infection, but the variety is sufficiently resistant to escape injury in the field. Apparently Webster reacts similarly.

From unpublished results obtained by Helen Hart,[4] it seems unlikely that Webster will be injured severely by stem rust, because its resistance seems to be due, at least partially, to the fact that there is a large amount of sclerenchyma in the stem. The collenchyma bundles are small. Consequently the rust is limited to small areas and cannot cause much injury. Hursh (4) recently has discussed in detail this type of resistance.

REACTION OF WEBSTER TO RUST IN THE FIELD

Webster was grown in the Minnesota rust nursery for the three years, 1915 to 1917, inclusive. The percentages of stem rust were as follows: 1915, 20 per cent; 1916, 65 per cent; 1917, 70 per cent with 50 per cent on the peduncles. These notes were taken by Messrs. J. H. Parker and O. S. Aamodt, who were in charge of the nursery during those years. Mr. McFadden observed that Webster was more resistant than Kota in six of the eight years during which he has had it under observation in South Dakota. It also has been resistant to orange leaf rust in the field.

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[4] Unpublished results of cooperative investigations between the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Minnesota.

REACTION TO ORANGE LEAF RUST

Webster also was inoculated in the greenhouse with fifteen collections of *Puccinia triticina,* including at least four or five physiologic forms. These collections were obtained from Minnesota, North Dakota, Missouri, Kansas, and Texas. Webster was very resistant to some collections and moderately susceptible to others. The type of infection varied from 0 to 4 –. Nine of the collections produced a type 3 infection; two, 3 + to 4 –; two, 1 =; and two caused flecks only. It seems likely, therefore, that Webster may be valuable also as a parent in breeding for resistance to the orange leaf rust.

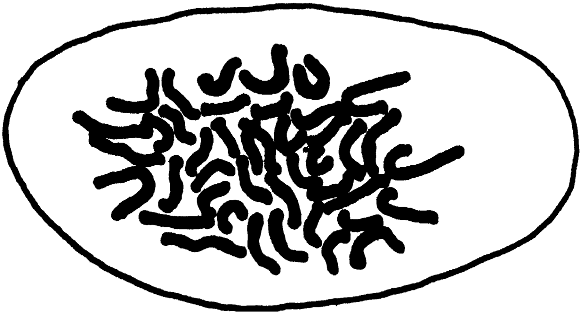

FIG. 1. Webster, C. I. 3780. Camera lucida drawing of a root-tip cell. × 1700. The chromosome number was found to be 42 in the somatic cells.

CHROMOSOME NUMBER OF WEBSTER WHEAT

Wheat species have been placed in three groups on the basis of taxonomic relationships by Schulz (10), and in similar groups by Tschermak (13), as a result of a study of genetic relationships. More recently, as mentioned above, the species have been placed in similar groups on the basis of their chromosome numbers (5, 8). Usually wheat varieties can be placed in one of the three groups with certainty on an inspection of morphologic characters. A variety occasionally is found, however, which is apparently intermediate between the durum and vulgare types, as is the case with Sevier wheat (1, 12), for example. Webster, while a common wheat in most characters, has a more pronounced keel than most of them and is somewhat spelt-like in appearance. In crosses made with common wheats by Mr. McFadden, the Webster variety behaved as a common wheat. As the chromosome number of a variety may be considered a fairly accurate criterion of its genetic relationships, it seems desirable to record the chromosome number for any new discoveries which are of interest from the breeding standpoint.

The chromosome number of Webster was determined from a study of root tip sections. The root tips were killed with modified Bouin's killing fluid, commonly known as B–15. They were carried through in the usual manner and imbedded in paraffin. Transverse sections were cut at a thickness of 10 microns. The sections were stained with Haidenhain's iron-alum haematoxylin. In good preparations it is a simple task to differentiate types with 28 chromosomes and 42 chromosomes. It is more difficult to determine accurately the actual number of a variety which can be placed at approximately 42. Counts were made of several cells and three camera lucida sketches were made from the cell, represented in figure 1. In all cases division figures were selected which apparently contained all of the chromosomes entire. That is, neighboring sections were scanned carefully to note the presence or absence of pieces of chromosomes which might have been cut from the figure being drawn. Counts were made only from cells in which the chromosomes were intact. The number of chromosomes was found invariably to be forty-two.

SUMMARY

1. Webster, C. I. 3780, a recently named variety of common wheat, *Triticum vulgare,* appears to be resistant to more physiologic forms of *Puccinia graminis tritici* than any other common wheat now known.

2. E. S. McFadden, who has grown Webster experimentally for several years in South Dakota, first called attention to its rust resistance.

3. Webster is not a desirable type of hard red spring wheat for commercial growing.

4. Webster probably will be valuable in breeding for rust resistance, because it is resistant to some physiologic forms of *P. graminis tritici* to which no other common wheat is known to be resistant. In attempts to produce desirable, rust-resistant wheats, resistant varieties of durum and emmer have been crossed with common wheats. However, there is some linkage between durum and emmer characters and rust resistance. And, owing to chromosome incompatibility, the percentage of sterility is high, necessitating the use of large numbers of plants. This makes the work of developing resistant varieties rather difficult.

5. Webster has been inoculated with nineteen physiologic forms of *P. graminis tritici* and is relatively resistant to all of them. It is resistant to five physiologic forms to which no other variety of common wheat is known to be resistant.

6. Apparently the rust resistance of Webster is due to the large amount of sclerenchyma in proportion to collenchyma in the stem. For this reason it seems likely that it will be resistant to all physiologic forms of *P. graminis tritici.*

7. There are only three physiologic forms of *P. graminis tritici* so far found in North America to which no *vulgare* wheat is known to be resistant. Webster has not yet been inoculated with these three forms.

8. If Webster is resistant to the three forms mentioned above, it will be possible to use *vulgare* wheats entirely as parents in crosses for rust resistance.

9. Webster has forty-two chromosomes and therefore is classed as a common wheat, although the spike is somewhat spelt-like and the glume has a well developed keel.

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THE REACTION OF SELFED LINES AND CROSSES OF MAIZE TO USTILAGO ZEAE[1,2]

F. R. IMMER AND J. J. CHRISTENSEN

INTRODUCTION

It has been learned from previous investigators (1, 2, 3) that selection in self-fertilized lines can be used to isolate lines of maize which differ markedly in their manner of reaction to *Ustilago zeae* (Beckm.) Ung. It was apparent also that it was comparatively easy to obtain selfed lines which were homozygous, or nearly so, and which were either highly resistant, highly susceptible, or intermediate in their reaction. The present paper gives the results of an investigation, which is a continuation of studies previously reported, on the mode of reaction to smut in the F_1 and F_2 generations and in backcrosses where selfed lines of known inheritance were used as parents.

EXPERIMENTAL RESULTS

The probable error method was used to determine what differences could be considered significant. The probable error in percentage for the amount of smut infection of two plots systematically replicated, as calculated by the pairing method (4), was determined for the groups of strains with a percentage of infection between two arbitrary limits. The probable error of each such class, or group of strains, and the number of pairs in the class from which the probable error was calculated are given in table 1.

TABLE 1.—*The probable error in percentage for smut infection of selfed strains and crosses differing in percentage of smut, as calculated by the pairing method*

Total smut in class, per cent	Pairs in class	Probable error, per cent
0.0 to 10.0	7	53.74
10.0 to 20.0	14	26.89
20.0 to 30.0	19	14.36
30.0 to 40.0	9	13.99
40.0 to 50.0	12	7.45
50.0 to 60.0	9	10.11
60.0 to 100.0	11	5.23

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[1] The authors wish to express their appreciation for the helpful suggestions of Drs. H. K. Hayes and E. C. Stakman in planning and conducting this investigation.

[2] Published with the approval of the Director as Paper No. 551 of the Journal Series of the Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minn.

The probable error in percentage decreased rapidly as the percentage of smut infection in the strains increased. While the actual deviation from the average in the low smut strains was smaller than in the high smut strains, the deviation in percentage was far greater in the low than in the high smut strains. The probable error for the entire experiment, as calculated by the pairing method, was 17.1 per cent. It is obviously unfair to use this percentage as the probable error for each strain, regardless of the percentage of smut, because such a probable error would be too low for the low smut strains and too high for the high smut strains. The probable errors of the strains in the 1924 test were calculated, therefore, according to the percentage of probable error for the class into which they fell, as given in table 1. The probable errors for the previous years, when used, were those reported by Hayes et al. in 1924.

REACTION OF PARENT LINES

Eight parent lines, which had proven to be apparently homozygous for a particular type of smut reaction under artificially induced epidemic conditions, were used to make the crosses for this inheritance study. The varieties Minnesota No. 13, Rustler, and Longfellow were used, and all crosses were made within a variety. The percentage of total and of ear smut infection of these parent lines for the years 1921 to 1924, inclusive, is given in table 2. Ear smut was calculated separately, because of its economic importance.

In general, the lines produced a relatively uniform percentage of smut from year to year. Since smut is due to a disease-producing organism which is dependent on environmental conditions for dissemination of the spores and consequent chance for infection, it is to be expected that there will be some variability in the percentage of smut produced. These parent lines were classified as high, medium, and low smut strains, depending on the average percentage of infection. Thus, the lines with an average of 0 to 15 per cent of total smut infection were classed as low smut strains; the lines with an average infection between 15 and 50 per cent of smut were classed as medium; and those with an average of over 50 per cent total smut infection were classed as high smut strains. While varying somewhat in percentage of smut from year to year, these parent lines quite uniformly stayed within their class limits.

REACTION OF CROSSES

The F_2 and back-crosses were made in 1923. In eight of the back-crosses it was possible to compare the percentages of smut infection of the progeny from crosses in which the F_1 was used as the male and from those

TABLE 2.—*The percentage of total and ear smut infection of self-fertilized strains of corn which were used as parent lines for subsequent crosses*

Variety	Cult. No.	Per cent of total smut					Per cent of ear smut				
		1921	1922	1923	1924	Average	1921	1922	1923	1924	Average
Minnesota No. 13	15	58.0	62.8	85.5	74.4	70.2 ± 3.9	32.0	26.6	16.8	36.5	28.0
" "	17	10.5	65.6	14.0	......	30.0 ± 2.2	0.0	0.0	2.0	......	0.7
" "	19	14.0	40.0	21.8	29.4	26.3 ± 1.7	0.0	4.0	4.5	0.0	2.1
Bustler	21	84.5	100.0	98.0	83.8	91.6 ± 5.0	11.2	21.3	28.0	56.0	29.1
"	23	28.5	2.5	12.1	33.4	19.1 ± 1.2	14.4	0.0	4.0	8.0	6.6
"	26	2.0	2.2	12.6	4.3	5.3 ± 0.8	0.0	0.0	0.0	0.0	0.0
Longfellow	46	4.5	19.4	7.4	15.0	11.6 ± 1.0	0.0	2.5	0.0	0.0	0.6
"	48	65.0	84.0	67.1	27.3	60.9 ± 4.0	12.4	6.0	30.6	5.5	13.6
"	51	12.0	27.7	69.1	52.4	40.3 ± 2.4	4.8	9.7	13.0	47.6	18.8

in which the F_1 was used as the female parent. A comparison of the results of these reciprocal crosses is given in table 3.

TABLE 3.—*A comparison of the percentage of total smut in comparable crosses when the F_1 was used as the staminate and when used as the pistillate parent*

Cross	Per cent smut		
	F_1 as ♂	F_1 as ♀	F_1 as ♂ minus F_1 as ♀
(Low x Med.) x Low	9.5 ± 5.1	6.1 ± 3.3	+ 3.4 ± 6.1
(Low x High) x Low	18.7 ± 5.0	26.2 ± 3.8	— 7.5 ± 6.3
(Low x Med.) x Med.	9.6 ± 5.2	10.8 ± 2.9	— 1.2 ± 6.0
(Low x High) x High	72.8 ± 3.8	53.0 ± 5.4	+ 19.8 ± 6.6
(Med. x Med.) x Med.	32.9 ± 4.6	30.0 ± 4.2	+ 2.9 ± 6.2
(Med. x Med.) x Med.	22.8 ± 3.3	22.5 ± 3.2	+ 0.3 ± 4.6
(Med. x High) x Med.	55.0 ± 5.6	45.2 ± 3.4	+ 9.8 ± 6.6
(Med. x High) x High	74.0 ± 3.9	74.3 ± 3.9	— 0.3 ± 5.5

The difference between each of the above crosses and its reciprocal was within three times the probable error. In 7 of the 8 comparisons the differences did not exceed one and one-half times the probable error. It may therefore be safely concluded that the factors for smut resistance or susceptibility are transmitted in the same manner in both male and female gametes.

In table 4 is summarized the percentage of total and of ear smut infection of the parent lines and of the F_1 and F_2 crosses of these lines.

In the F_1 crosses the percentage of smut infection tended to be intermediate. In the cross between the strains classed as medium (17 x 19), and between the medium and low strains (23 x 26), the F_1 was somewhat less severely infected than either parent. It appears possible that infection is somewhat less in vigorous crosses than in selfed lines, although it is also evident that smut reaction is dependent on definitely transmissible genetic factors. With a complete, or almost complete, lack of dominance it is to be expected that the F_2 would produce approximately the same percentage of smut infection as the F_1. In 4 of the 6 crosses, the difference between the F_1 and F_2 was less than twice its probable error, and in all crosses the difference was less than three times its probable error.

In none of the 19 backcrosses was the percentage of smut infection obtained significantly larger or smaller than that of the average of the parents. It may be safely concluded from these data that there is no definite dominance of resistance or susceptibility to smut infection.

In studying the inheritance of reaction to ear smut, it is found that three of the F_1 crosses were more severely, and three less severely, infected than the average of the parents. Five of the six F_2 crosses produced a higher percentage of ear smut infection than did the F_1. Of the 19 backcrosses, 10 produced a slightly higher percentage of smut than either parent, and the remaining 9 were intermediate in reaction.

TABLE 4.—*A summary of the reactions of parent lines and of F_1 and F_2 crosses to attacks of Ustilago zeae*

Variety	Parent line or hybrid	Class for smut reaction of parents	Generation selfed or cross	Per cent total smut	Per cent ear smut
Minn. No. 13	17	Medium	5	30.0 ± 4.2	0.7
" "	19	Medium	5	26.3 ± 3.7	2.1
" "	17 x 19	Med. x Med.	F_1	20.6 ± 3.0	3.3
" "	17 x 19	Med. x Med.	F_2	26.2 ± 3.8	7.7
Minn. No. 13	15	High	5	70.2 ± 3.7	28.0
" "	17	Medium	5	30.0 ± 4.2	0.7
" "	15 x 17	High x Med.	F_1	45.8 ± 3.4	17.3
" "	15 x 17	High x Med.	F_2	52.2 ± 5.3	22.1
Rustler	21	High	5	91.6 ± 4.8	29.1
"	26	Low	5	5.3 ± 2.8	0.0
"	21 x 26	High x Low	F_1	25.0 ± 3.6	4.5
"	21 x 26	High x Low	F_2	34.4 ± 4.8	11.1
Rustler	23	Medium	5	19.1 ± 5.1	6.6
"	26	Low	5	5.3 ± 2.8	0.0
"	23 x 26	Med. x Low	F_1	2.8 ± 1.5	0.0
"	23 x 26	Med. x Low	F_2	12.9 ± 3.5	2.4
Longfellow	46	Low	5	11.6 ± 3.1	0.6
"	48	High	5	60.9 ± 3.2	13.6
"	46 x 48	Low x High	F_1	61.0 ± 3.2	17.0
"	46 x 48	Low x High	F_2	47.5 ± 3.5	12.0
Longfellow	46	Low	5	11.6 ± 3.1	0.6
"	51	Medium	5	40.3 ± 3.0	18.8
"	46 x 51	Low x Med.	F_1	24.7 ± 3.5	6.5
"	46 x 51	Low x Med.	F_2	16.8 ± 4.5	10.5

REACTION OF LOW SMUT STRAINS SELECTED UNDER NORMAL FIELD CONDITIONS WHEN TESTED UNDER SMUT EPIDEMIC CONDITIONS

Fourteen selfed lines of corn which had produced a low percentage of total smut in the regular corn breeding nursery for several years were grown in the smut inheritance plot in 1924. The purpose of this was to test the reliability of selection of low smut strains under normal field conditions when compared with their reaction under artificially induced smut epidemic conditions. The results are given in table 5.

TABLE 5.—*The percentage of smut infection of strains of corn which had given a low smut infection for several years in the regular corn-breeding nursery*

Cult. No.	Variety	Per cent smut infection	
		Total	Ear
73	Minnesota No. 13	2.0 ± 1.1	0.0
74	"	43.2 ± 3.2	3.9
75	"	26.3 ± 3.8	13.2
76	Rustler	12.2 ± 3.3	0.0
77	"	13.0 ± 3.5	0.0
78	"	71.1 ± 3.7	7.9
79	"	10.2 ± 2.7	4.1
80	"	48.8 ± 3.6	5.0
81	"	11.7 ± 3.1	1.3
82	Northwestern Dent	4.1 ± 2.2	0.0
83	"	100.0 ± 5.2	12.5
84	"	24.6 ± 3.5	3.1
85	Longfellow	40.0 ± 3.0	22.0
86	King Phillip	53.1 ± 5.4	12.5

Of these 14 selfed lines selected as low smut strains under ordinary field conditions, 6 would be classed as low, 5 as medium and 3 as high smut strains under epidemic conditions. On the basis of this single year's test it may be concluded that selection of low smut strains under natural field conditions is an aid to obtaining smut-resistant strains, but that they should be tested under epidemic conditions in order to be sure of their resistance.

THE INHERITANCE OF A FIRING CHARACTER IN A CROSS OF TWO SELFED LINES OF MINNESOTA NO. 13

A rather striking firing character had been observed and notes taken on it for several years, previous to 1924, in one of the Minnesota No. 13 strains (Culture 15). Other strains growing beside this firing strain were observed to be free from this condition. This firing made itself manifest by a drying up of parts of the leaves, especially at the tips. Long, slender blotches appeared on the leaves and seemed to follow the vascular bundles, indicating an intervascular type of firing. The first symptoms were several times suspected of being due to a pathological condition. Cultures were made but no organism was found. Since chlorophyll abnormalities in maize seedlings often give similar long, slender blotches on the leaves, it is suggested that this condition may be another mature plant chlorophyll abnormality.

The parent line of Minnesota No. 13, culture 15, had 76 per cent of the plants fired in 1921 and all plants fired in 1922, 1923, and 1924. Another parent line of Minnesota No. 13, culture 17, showed no fired plants in 1921 or 1922 and only a few plants fired in 1923. The season of 1923 was very dry, and the drying up of some of the leaves was probably not due to the same genotypic condition that caused the firing in culture 15. No record of culture 17 was available in 1924 because of loss of the strain. These two lines, which also differed in smut reaction, were crossed in 1922. The F_1, F_2, and backcrosses were grown in 1924. Notes were taken on the individual plants, and the degree of firing was classified as heavy, medium or none.

The firing parent had 68 plants heavily fired and 6 plants medium fired in 1924. The observed numbers of plants in the different firing classes, and the numbers which would be expected on the basis that a single factor in the homozygous recessive condition determined heavy firing, in the heterozygous condition determined medium firing and in the homozygous dominant condition determine no firing, are given in table 6.

TABLE 6.—*Observed number of plants differing in degrees of firing, and the calculated number, on the basis of a single factor difference, in a cross between a firing and a non-firing selfed strain*

Cross	Observed or calculated	Number of plants			
		Degree of firing			Total
		Heavy	Medium	None	
F_1	Observed	5	162	4	171
(All × none)	Calculated	0	171	0	171
F_2	Observed	172	333	158	663
(All × none)	Calculated	166	333	166	663
Backcross	Observed	141	170	43	354
(All × all × none)	Calculated	177	177	0	354
Backcross	Observed	26	243	143	412
(None × all × none)	Calculated	0	206	206	412

The F_1 showed a complete lack of dominance, the few plants classed as heavily fired and as non-fired being no more than would be expected from the normal variability of such a character and from the fact that slight errors may have been made in taking notes.

Segregation took place in F_2. The "goodness of fit" method of calculating the deviation from the expected, on the basis of a single factor difference, gave the results shown in table 7.

With $X^2 = .606$, P would be very large and the chances would be very great that the deviation from the expected was due to chance variability.

The deviation in the backcrosses from expectation on a single factor basis was rather large. It is apparent, however, that the condition is not very complex from an inheritance standpoint, although more than a single genetic factor may be involved.

TABLE 7.—*Comparison by the "goodness of fit method" between observed and calculated number of plants in the F_2 of a cross between firing and non-firing strains*

Degree fired	Number of plants		O–C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
Heavy fired	172	166	6	36	.217
Medium fired	333	332	1	1	.003
Non-fired	158	166	8	64	.386
				$X^2 =$	.606

LINKAGE BETWEEN FIRING AND SMUT REACTION

In table 8 is summarized the correlation between firing and smut reaction. The F_1 cross was made in 1922 and the other crosses were made in 1923. All crosses were grown in 1924.

TABLE 8.—*Correlation between reaction to smut and a firing character in crosses between a firing, high smut (70.2%), selfed strain and a non-firing, medium smut (30.0%), selfed strain, 1924*

Type of parent or cross	Fired			Not fired		
	No. Smutted	No. not Smutted	Per cent Smutted	No. Smutted	No. not Smutted	Per cent Smutted
All fired, high smut parent ..	55	19	74.3 ± 3.9			
F_1 (all fired, high smut × none fired, medium smut)	77	89	46.3 ± 3.4	2	2	50.0 ± 5.1
F_2 (all fired, high smut × none fired, medium smut)	274	231	54.3 ± 5.5	78	80	49.4 ± 3.7
F_1 × all fired, high smut parent	240	71	77.2 ± 4.0	22	21	51.2 ± 5.2
F_1 × none fired, medium smut parent	126	143	46.8 ± 3.5	64	79	44.7 ± 3.3

If there were a linkage between the factors determining firing and those determining smut reaction, it would be expected that the fired plants would have a higher percentage of smut than the non-fired plants. In only one cross, that of the F_1 with the all fired, high smut parent, was there a significantly higher percentage of smut on the fired than on the non-fired plants. The number of non-fired plants, however, was very small in this cross. In consideration of this and the fact that no linkage was evident in the F_2 or other backcross, it cannot be safely concluded that there is a linkage between the factors determining firing and smut reaction.

SUMMARY

1. The probable error in percentage decreased uniformly as the percentage of smut infection in the paired plots increased.
2. The parent lines developed a uniform percentage of smut from year to year (1922–1924 inclusive).
3. The factors determining resistance or susceptibility were transmitted in the same manner in both male and female gametes.
4. Dominance of resistance or susceptibility to smut reaction was lacking. Further study is necessary before concluding how many factors are involved.
5. Low smut strains of corn selected under normal field conditions should be tested under smut epidemic conditions in order to be sure of their resistance.
6. The inheritance of firing can be adequately explained on the basis of a single factor difference. There was apparently no correlation between smut and firing.

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TWO UNDESCRIBED SPECIES OF BOTRYTIS ASSOCIATED WITH THE NECK ROT DISEASES OF ONION BULBS[1]

J. C. WALKER

WITH TWO FIGURES IN THE TEXT

The neck rot diseases of onion have been the subject of study by the writer since 1917, particularly as they occur in the onion-growing sections in the middle west. In that year Munn (1) published the results of his studies upon a neck rot disease in Michigan and New York, in which report he described a new species, *Botrytis allii*, as the causal organism. This form has been found in the Wisconsin and Illinois sections, and his findings with regard to the morphology and pathogenicity of the organism have in the main been corroborated. It became evident early in the course of the writer's investigations that two other distinct species of *Botrytis* are commonly associated with this disease and one of these forms is by far the most important cause of neck rot in the Wisconsin and Illinois sections. A careful study of the two last forms has shown that they are apparently undescribed species. Since each of the three species causes symptoms upon the host which though very similar to those of the other two forms are nevertheless distinct, it seems appropriate to designate each disease separately. The writer therefore has chosen to speak of them subsequently as (1) the grey-mold neck rot caused by *Botrytis allii* Munn, (2) the mycelial neck rot caused by *Botrytis byssoidea* n. s., and (3) the small sclerotial neck rot caused by *Botrytis squamosa* n. s. The mycelial neck rot causes greatest losses of all three diseases in the Wisconsin and Illinois sections. The small sclerotial neck rot, found so far only upon white varieties, is confined more particularly to the dry outer scales of the bulbs and is therefore least destructive of the three diseases. Certain physiological studies of one or another of the three forms already have been published (3, 4). In these reports the mycelial neck rot form has been referred to as *Botrytis sp. 110*, and the small sclerotial neck rot form as *Botrytis sp. 108a*. The control of mycelial neck rot by artificial curing also has been previously discussed (2). A more detailed discussion of the pathological phases of the three diseases will be published in another paper. Accounts of the two undescribed species are presented at this time.

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[1] The writer is indebted [illegible] Prof. H. H. Whetzel, of Cornell University, for advice furnished during the prep[illegible]on of this manu

Botrytis byssoidea n. s. (Fig. 1.)

Mycelium hyaline, septate, variable in diameter, branches sometimes constricted slightly at the base; conidiophores arising directly from the mycelium or from sclerotia, erect, becoming flattened and twisted with age,

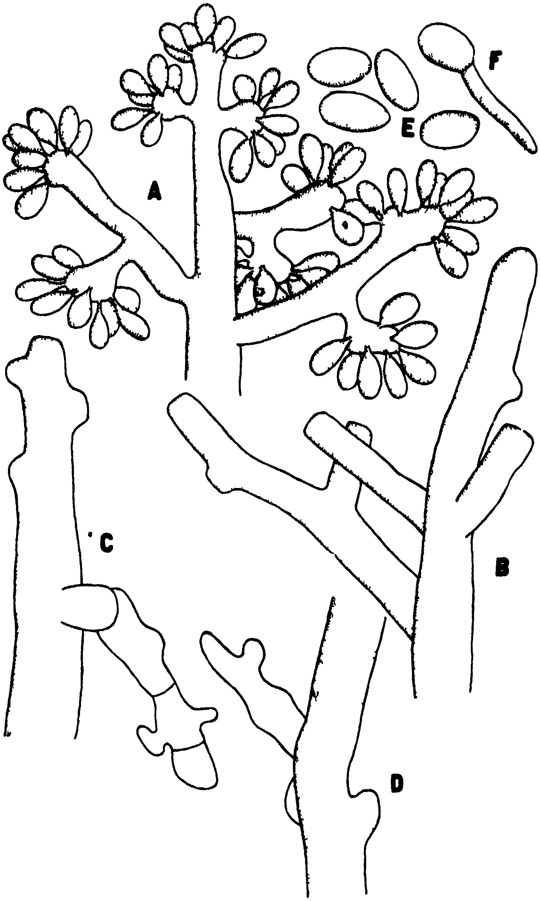

FIG. 1. Conidial production of *Botrytis byssoidea*.

A. Tip of the conidiophore showing characteristic branching and the production of conidia upon short sterigmata which arise from the rounded apices of the branches. B. Tip of conidiophore some time after sporulation; the main stalk is proliferating, while the sporiferous tips of the branches have been cut off by septa and have disappeared; the branches continue to degenerate. C. Conidiophore in which the sporulating side branches have all degenerated, having been cut off by septa near the main stalk and leaving slight projections. D. Remains of sporulating branches on a conidiophore which has proliferated and grown on to produce more sporulating branches at the growing tip. E. Mature conidia. F. Conidial germination in tap water after 20 hours at 20–22° C. × 600.

hyaline at the growing tip, walls becoming deep brown with age, septate, slightly swollen at the base, occasionally branched, branches constricted at the base; hyaline sporiferous branches, formed at the growing tip, rebranch and produce the conidia upon short hyaline sterigmata on their rounded apices; these branches occasionally proliferate, but usually degenerate as soon as the conidia are mature, having been cut off close to the main trunk by septa; the main trunk proliferates and is marked by scars or short projections at the points where sporiferous branches have degenerated. Conidia obovoid, smooth, continuous, hyaline, ashen grey in mass when young, becoming somewhat darker with age; sterigmata not often remaining attached; 8–19 × 5–11 μ (mostly 10–14 × 6–9 μ). Microconidia globose, about 3 μ in diameter, borne upon short, hyaline, conidiophores. Sclerotia white at first becoming black with age; raised and rounded on the upper surface, flat or concave on side in contact with the host, variable in size (1 to 5 mm. or more), irregular in shape, often converging into large conglomerates; in cross section, pseudoparenchymatous, several outer layers of cells dark walled, interior hyaline; germination under favorable conditions by hyaline hyphae or by conidiophores.

Parasitic on bulbs of *Allium cepa,* appearing usually after harvest and infecting usually at the "neck" of the bulbs; distribution, Wisconsin, Illinois, Connecticut, France. Type specimens deposited with Office of Pathological Collections, Bureau Plant Industry, U. S. Department of Agriculture, Washington, D. C.; Herbarium of the University of Wisconsin, Madison, Wis ; and Herbarium of Royal Botanic Gardens, Kew, Surrey, England.

Botrytis squamosa n. s. (Fig. 2.)

Mycelium hyaline, septate, variable in diameter, branches not ordinarily constricted at the base. Conidiophores comparatively rare at 20–22° C, more abundant at cooler temperatures; seldom rising directly from the mycelium, more often in tufts from sclerotia; erect, becoming flattened and twisted with age; hyaline at first, turning dark with age; septate, slightly swollen at the base; branches common and constricted at the base; growing tips branch and rebranch previous to sporulation; conidia borne on short hyaline sterigmata arising from swollen apices of branches and become detached at maturity; side branches degenerate after fructification, the walls drawing back in characteristic accordion-like folds; degenerating side branches cut off by septa laid down near the base, leaving distinct scars or knobs upon the main stalk; main stalks proliferate and sporulate repeatedly depending upon conditions. Conidia obovoid to ellipsoid, smooth, continuous, hyaline, ashen grey in mass when young becoming somewhat darker with age; sterigmata seldom remaining attached; 13–22 × 10–17 μ, mostly 15–20 × 12–15 μ. Microconidia globose, about 3 μ in diameter, borne upon short, hyaline conidiophores. Sclerotia white at first, turning black with age; most common on dry outer scales of the host, roughly circular in outline, flat, scale-like, 1/2 to 4 mm. in diameter, rarely more than 1/8 mm. in thickness; often converging into large scale-like conglomerates.

Parasitic on outer scales of bulbs of *Allium cepa,* especially upon white varieties; distribution, Wisconsin, Illinois. Type specimens deposited with the Office of Pathological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.; Herbarium of the Univer-

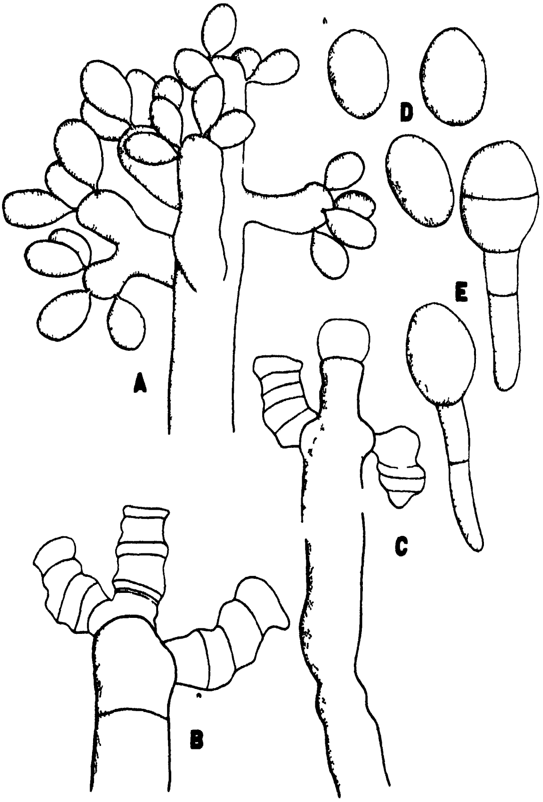

FIG. 2. Conidial production of *Botrytis squamosa;* the process is very much like that of *Botrytis byssoidea.*

A. Sporulating tip of conidiophore. B. Degenerating sporiferous branches which shrink back in accordion-like folds. C. Conidiophore showing degenerating branches at the tip, while lower down are the irregularities left from the previous group of sporiferous branches. D. Mature conidia. E. Conidial germination in tap water after 27 hours at 20–20° C. × 600.

sity of Wisconsin, Madison, Wis.; Herbarium of the Royal Botanic Garden, Kew, Surrey, England.

The three species of Botrytis associated with onion neck rot are very easily distinguished by their characteristic types of growth upon a number of standard cultural media. Inasmuch as this may be used as a ready means of diagnosis, the salient characters of the growth of the three forms upon potato-dextrose agar plates at room temperature are given.

Botrytis allii.

Produces a rapidly growing colony reaching a diameter of about 50 mm. in 4 days at 20–22° C. In the beginning, the center of the colony consists of dense whitish aerial mycelium, while the outer zone of about 5 mm. in width consists of scanty, creeping extending mycelial threads. Conidiophores and conidia appear on about the third day, nearly white at first, turning smoky grey with age. Eventually the entire plate becomes covered with a dense uniform layer of conidiophores. Sclerotia are not commonly produced.

Botrytis byssoidea.

The colony grows somewhat faster at 20–22° C. than does that of *Botrytis allii.* Abundant white, fluffy, aerial mycelium forms which is more raised and cottony than that of either *Botrytis allii* or *Botrytis squamosa*; fairly uniform in density except at the very center where there is little aerial mycelium, and at the narrow outer zone of extending hyphae. Conidia are seldom produced. Sclerotia absent.

Botrytis squamosa.

The colony which enlarges more slowly than either *Botrytis allii* or *Botrytis byssoidea* consists of white fluffy aerial mycelium, except for the outer advancing zone, but it is less raised and fluffy than that of *Botrytis byssoidea.* Abundant sclerotia appear over the entire plate after two to three weeks, first as dense whitish mycelial masses which become hard, and black with age; mostly 1–2 mm. in width and thicker and more rounded on the upper surface than those which develop upon the host. There is very little sporulation at room temperature (20–22° C.) but profuse production of conidiophores arising directly from the sclerotia occurs at 12° to 16° C.

OFFICE OF COTTON TRUCK AND FORAGE CROP DISEASE INVESTIGATIONS,
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A NOTE ON THE BROWN LEAF-SPOT OF ALFALFA

L. R. TEHON AND EVE DANIELS

WITH ONE FIGURE IN THE TEXT

During each of the years 1922 and 1923, the collections made by the botanists of the Illinois State Natural History Survey included a few specimens of alfalfa leaves affected by a marked browning which, in its more extensive manifestation, assumed the character of a severe leaf blight. These were set aside under the suggestive label *Macrosporium sarcinaeforme* Cav. In 1924 numerous additional specimens of the same kind were collected from widely separated parts of Illinois. The nature of the diseased spots and the notes accompanying the specimens indicated that the disease was of some importance, and that it was responsible for a considerable loss in the fields in which it was found.

The fact that the fungus has been reported several times as causing severe losses upon red clover in the United States and in Europe suggests that its presence upon alfalfa may constitute another serious impediment to the successful production of that very valuable crop, at least in certain seasons. An account of the disease and of the fungus, as we have seen it in Illinois, should therefore be of interest.

SYMPTOMS

The first symptom of this disease is a marked local yellowing of the leaf tissue. A dead area, brown in color, soon appears, replacing the yellowing which is apparent thereafter only as a narrow band of etiolated tissue marking the advance of the rapidly enlarging spot. Infection may take place at any point on the leaflet, although usually it is marginal. Intramarginal infections are circular and, for a time, quite arid. The extension of the spot is rapid, encompassing, as it spreads, whatever other lesions there may be upon the leaflet. Ultimately much of the tissue is involved.

The older portions of the lesions become dark brown and wrinkled, and eventually appear sooted from the abundant production of spores and hyphae by the fungus.

DISTRIBUTION AND IMPORTANCE

The first collection of the brown leaf-spot in Illinois was made July 14, 1922, in Shelby County, and the second, July 16, 1923, in Bond County—both in the southern half of the state. During the summer of 1924 it was seen in many fields, first in Jersey County, August 25, and last in Carroll

County, September 17. With the exception of the first, all the 1924 records came from the northern half of the state. The accompanying map (Fig. 1, a) shows the counties in which it has been found, and suggests that it is even more widespread. So extensive a distribution here indicates the probability of its occurrence in neighboring states as well. Indeed, Dr. F. R. Jones writes that he has seen this disease occurring extensively in northern states, practically from the Atlantic coast to Minnesota.

Like other leaf diseases of alfalfa, this one causes its chief damage by killing the leaflets and causing them to shatter off, thus reducing the harvestable crop. As observed in Illinois, the attack is general in infested fields, and the damage done is often directly in proportion to the number of diseased leaflets per plant. Table 1, compiled from the 1924 field notes, indicates the ranges of infection observed and shows that the disease may be, under proper circumstances, either very light or very serious. It is significant that in the fields examined the acre-average shows that about 20 per cent of the leaves were infected.

TABLE 1.—*Summary of field notes on the prevalence of the brown leaf-spot of alfalfa in Illinois*

County	Acres	Per cent of diseased plants	Per cent of diseased leaflets
Boone	5	100	33
Carroll	4	100	2
Cook	6	100	1
Jersey	10	100	1
Lake	4	100	75
do	10	100	33
Peoria	2	100	10
Putnam	2	100	25
		Acre-average	20.67

Macrosporium sarcinaeforme was originally described by Cavara (2) from Italy in 1890. The earliest record of its occurrence in the United States is a specimen collected by May Varney (No. 1193) at Manhattan, Kansas, October 12, 1889, on clover. Malkoff (5) records it as a serious leaf blight of clover in Europe. Orton (6) reports that its prevalence upon clover in Connecticut and New York caused comment in 1903, and that (7) it was common in Tennessee in 1905, but subordinate to anthracnose. Bain and Essary (1) record it as being destructive on red clover and occasionally on alsike in Tennessee. The only reference to the presence of the fungus on alfalfa of which the writers are cognizant is one by Westgate (12), who speaks of it casually in a brief discussion of alfalfa diseases: "Another form of leaf spot (*Macrosporium sarcinaeforme*) is sometimes destructive This appears in the form of well defined circular spots which show numerous black dots scattered over their surfaces"

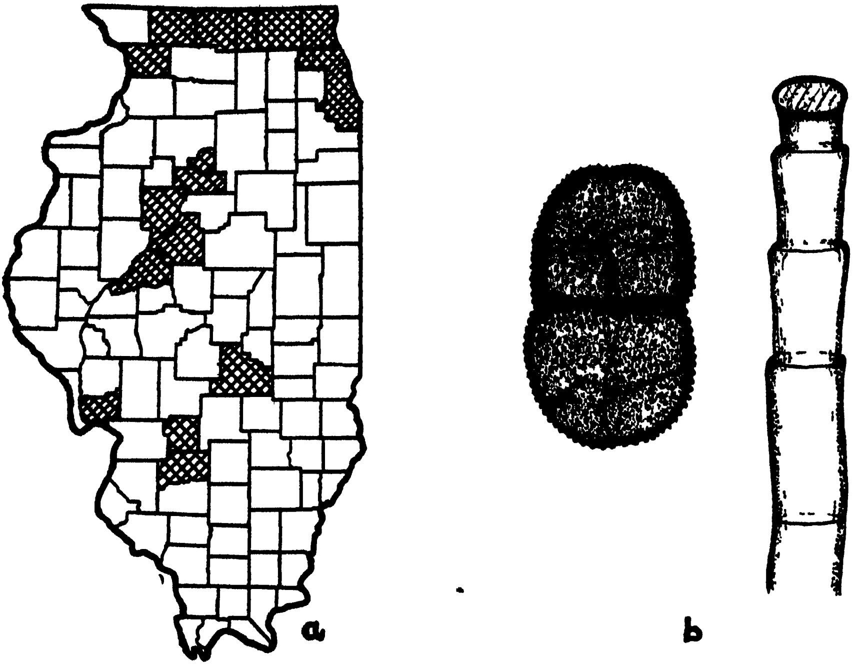

FIG. 1. a. Known occurrence of the brown leaf spot of alfalfa in Illinois (Indicated by crosshatched areas) b A spore, and the apex of a conidiophore, of *Thyrospora sarcinaeforme*.

TAXONOMY

Comparison of the alfalfa parasite with the Varney specimen and with available exsiccati reveals no striking differences such as would justify specific segregation. As we have seen it, the fungus has upright conidiophores which are either single or grouped in fascicles. The fascicles are not large; rarely are there more than four hyphae. The olive-brown conidiophores are short—much shorter than is recorded in Malkoff's diagnosis of the fungus as it occurs on clover. Their nodulose character, noted both by Cavara and Malkoff, gives them a striking, jointed appearance which seems to result from the continued apical growth of the hyphae following the individual abscissions of successively formed spores.

The spores, which are borne singly upon the apices of the conidiophores, are olive-brown, co[illegible]orous, and muriform, with a marked median constriction at the heavy lateral septum which divides the spore into slightly unequal halves (Fig. 1, b). The epispore is definitely, but minutely, echinulate. There is considerable variation in size and septation among the spores, but the measurements, both of length and width, fall well within the limits prescribed by Cavara and those observed by Malkoff, while the variation in septation is concerned only with the number of diagonal walls in the spore and not with the lateral septum.

Macrosporium medicaginis Cugini (11) apparently causes a very similar lesion on alfalfa leaves; and the obvious difficulties of specific determination in *Macrosporium* at once raises the question as to whether our alfalfa parasite may not be identical with the one described by Cugini. There can be no doubt that the two are distinct. Cugini's figures illustrate the usual oblong spores of the genus, with the customary type of septation, and show conidiophores made up of pyriform (as the description says) or (more accurately) torruloid compartments. Cugini states that his species approaches *M. meliloti* Pk.; and Traverso adds, in a footnote, that it also approaches *M. globuliferum* Vestergr. Peck's (8) description of his species, while not altogether clear, is definite with respect to the spores, which are "subelliptical, or clavate, generally tapering below into a short pedicel, 3–5 septate with a few longitudinal septa"—a description not in any way applicable to ours, but confirming our belief that we are dealing with *M. sarcinaeforme.* Vestergren's (9) description of *M. globuliferum* speaks of typically 3-septate muriform spores slightly constricted at the septa. These would not be like the spores we have. The "subquadratic" divisions of the conidiophores of *M. globuliferum* are said by Traverso to be one of the points of difference between it and *M. medicaginis,* and he thereby emphasizes a marked distinction between Cugini's species and the one with which we are dealing.

It would appear, therefore, that the fungus we have found on alfalfa in Illinois is identical, so far as may be judged from descriptions and available figures and specimens, with *M. sarcinaeforme.*

The original definition of the genus *Macrosporium* is not sufficiently clear, either as to its wording or with respect to its original species, to permit definitely of the inclusion of sarcinaeform-spored types. Elliott (3) definitely excludes them and suggests that they may be placed in the genus *Stemphylium;* but his suggestion is not applicable to our fungus, since the definition of that genus requires repentiform conidiophores.

Among the Fungi Imperfecti it is a tenable procedure to make distinctions, greater than specific ones, partly upon the character of the epispore · in fact, satisfactory grouping often makes it necessary. Thus, *Macrodiplodia* is separated from several other diplodioid genera by the mucus covering of its spores. The same is true of *Cylindrocephalum* and *Acontium*. The number of genera separated on the basis of spore ciliation is large. A spiny epispore distinguishes *Roumegueriella* from *Zithia* and related genera, and *Zygodesmus* from three others; verrucose spores separate *Discomycopsella* from *Pirostoma;* and echinulate spores separate *Heterosporium* from *Helminthospora* and *Helminthosporium*.

Species of *Macrosporium* of the type represented by *M. sarcinaeforme* have been excluded from the genus by Elliott because of their sarcinaeform spores. The upright, unbranched conidiophores exclude them from *Stemphylium* also. The minutely echinulate epispore is a character not properly ascribed to either. It seems desirable, therefore, to provide a genus capable of including this and other similar species now incapable of proper generic reference; and for this purpose we are establishing the genus *Thyrospora*, the designation being in accord with the usage of Hoehnel (4) and of Sydow and Werdermann (10) for fungi of similar aspect.

Thyrospora gen. nov.

Dematiacea, dictyospora, macronemea. Hyphis erectis, septatis, singulis aut fasciculatis, coloratis. Conidiis muriformibus, sarcinaeformibus, echinulatis, gestis singillatim, ex apice hypharum oriundis, coloratis.

Spectat ad Thyrodochium Werd., genus Tuberculariacearum. Species typica:

Thyrospora sarcinaeforme (Cav.) Comb. nov.

Syn. *Macrosporium sarcinaeforme* Cav. Dif. dei Parass. 1890.

NATURAL HISTORY SURVEY,
URBANA, ILLINOIS.

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SCLEROSPORA ON CORN IN AMERICA

I. E. MELHUS AND FRANK VAN HALTERN

Sclerospora graminicola (Sacc.) Schroet. is very common in Iowa and adjoining states on *Setaria viridis* (L.) Beauv. (green foxtail), a prevalent and troublesome weed in corn fields.

This fact led the authors to try infection of corn with the downy mildew occurring on *Setaria*. Having had success in infecting *S. viridis* using oospores on the seed and in the soil, they attempted to infect corn. Infection took place within two days after the spores were placed on the seed under conditions favorable for their germination. Infection was readily obtained on dent, sugar, pop, and flint varieties. Infection takes place before emergence of the plumule from the soil. In many cases ninety per cent of the plants exposed became infected.

The symptoms on *Setaria* and corn are markedly different in some respects. On corn the symptoms are more often a grayish blotching and mottling that may extend throughout the whole plant. In other cases, only a few mottled yellow spots develop, or chlorotic areas occur in the form of longitudinal stripes apparently following between the vascular bundles of the blades.

Mottling may appear as irregular, isolated spots one-fourth inch to several inches long and may often develop quite rapidly in an apparently healthy seedling. Symptoms usually appear within ten days after the plumule emerges, but may not develop for three weeks. This apparently depends upon how soon infection occurs after germination.

Infected corn plants are almost always stunted, depending on the severity of the infection. Some may succumb and die when only three inches tall, while a few appear to outgrow the disease. Many infected plants fail to develop other chlorotic areas after the initial symptoms appear, but become very rich green and have a bushy, stocky appearance, due to the production of the normal number of leaves on a stalk with shortened internodes.

The dwarfing is much like that described by Weston,[1, 2] due to *Sclerospora Philippinensis* Weston and *S. spontanea* L. in the Orient. Conidial sporulation is rather sparse, while oospore production has never been found. Weston[1] also reports that he never found oospores of *S. Philip-*

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[1] Weston, W. H. Philippine downy mildew of maize. Jour. Agr. Res. 19: 97–122. 1920.

[2] Weston, W. H. Another conidial *Sclerospora*, of Philippine maize. Jour. Agr. Res. 20: 669–684. 1921.

pinensis on corn in the Philippines. Some plants may become quite downy with conidiophores, while others may produce few or none, although showing marked symptoms of the disease. The heaviest sporulation on corn is much less than the ordinary sporulation on *Setaria viridis* and occurs only in the seedling stage. This probably explains why no one has recorded the presence of this disease heretofore.

Soil from a field where the downy mildew was abundant the previous year on *Setaria viridis* was brought to the greenhouse at various times, and infection was secured on plants from foxtail seed planted in the soil and on volunteer plants. It is evident that the oospores overwinter in the soil and infect the germinating seed of foxtail early in the spring. From these centers the fungus may spread by means of the conidia. Experiments indicate that the oospores remain viable in the soil over a long period of time so that succeeding crops of foxtail may be infected from oospores as well as by conidia.

Sclerospora graminicola apparently has a wide host range It was readily transferred to two varieties of millet, *Setaria italica* (L.) Beauv. and *Panicum miliaceum* L, to Teosinte, *Euchlaena mexicana* Schrad. and to 26 varieties of *Zea mays* The varieties of pop corn are the most susceptible, Twelve varieties of dent corn were readily infected.

IOWA AGRICULTURAL EXPERIMENT STATION,
AMES, IOWA.

THE LITERATURE OF PLANT PATHOLOGY AND THE PLANT INDUSTRY CATALOG

NEIL E. STEVENS

So close has been the cooperation between the library of the Bureau of Plant Industry and the editorial boards of PHYTOPATHOLOGY, and so general has been the use of the facilities of the Bureau by members of the Phytopathological Society that the publication in this Journal of a sketch of the "Plant Industry Catalog" might seem superfluous. The recently retired editor has, however, called our attention emphatically to the increasing danger that important earlier work may be overlooked in preparing papers for publication. One of the best, perhaps the best, means of avoiding this danger is furnished by a good card catalog.

The "Plant Industry Catalog" consists—like ancient Gaul—of three parts, the author catalog, the general botany subject catalog, and the plant pathology catalog. The author catalog was begun in 1903 by Miss Marjorie F. Warner, but since 1906 has been maintained by Miss Alice C. Atwood, who inaugurated the general botany subject catalog. The plant pathology catalog was begun by Miss Oberly some time before 1908, but during 1919, when Miss Oberly was detailed to the Reclassification Commission, Miss Atwood took over the work on the plant pathology catalog and has carried it on since Miss Oberly's death in 1921.

Little need be said about the author catalog. It has no peculiarities of arrangement save that an attempt is made to bring the editions of a work together and also to have translations follow the original work, with cross references from varying titles. Biographical and bibliographical works about authors are included.

The general botanical subject catalog, which covers botany in its widest sense (systematic, economic, geographic, ecologic, morphological and physiological botany, etc.), is a semi-classed catalog, that is, the main headings are in alphabetical order but under these are many divisions and sub-heads. For instance, under IRRITABILITY AND MOVEMENT OF PLANTS are found as sub-heads, *Geotropism, Heliotropism, Twining of plants,* etc.; the large group FUNGI has many divisions and such headings as *Fungi, Nomenclature—Fungi, Edible and poisonous—Fungi, Morphology and physiology,* besides a geographic arrangement. The botany of the United States is under the heading UNITED STATES BOTANY, with large subdivisions (*New England—Western States,* etc.) arranged under it, followed by the states in alphabetical order. CITRUS FRUITS is used as a general heading with individual fruits brought out as sub-heads and the same arrangement obtains for SMALL FRUITS, NUTS and many other headings. These are only a few examples of the method of arrangement. Cross references enable one to trace a subject from its alphabetical position to its place in the semi-classed arrangement.

The plant pathology catalog deals with diseases of plants caused by fungi, bacteria, parasitic phanerogams and nematodes; physiological diseases and injuries; and diseases of the mosaic type. Like the general botany catalog, it is semi-classed. The main arrangement is by host, with subdivisions for *Disease resistance, Diseases* (in general), *Diseases, Control,* followed by the individual diseases as sub-heads in alphabetical order. Common names are used for the diseases whenever the common name is widely used; if it is not sufficiently well known the name of the organism is used. Besides these, there are sub-heads for diseases not caused by fungi or bacteria such as *Chlorosis, Mosaic, Physiological diseases,* etc. References are made from the name of the organism to the host, as will be shown by examples given below. A fifth subdivision under the host is *Injuries,* under which are included "Lightning injury," "Weather injuries," etc.

The following examples will show the general arrangement under host:

Celery. Disease resistance and resistant varieties.
Celery. Diseases.
Celery. Diseases. Control.
Celery. Diseases. Bacillus apiovorus.
Celery. Diseases. Bacterial leaf spot. Pseudomonas apii.
Celery. Diseases. Cercospora apii see Celery. Diseases. Late blight.
Celery. Diseases. Club root.
Celery. Diseases. Mosaic.

In their alphabetical place in the catalog will be found the references:

Bacillus apiovorus. See Celery. Diseases. Bacillus apiovorus.
Pseudomonas apii. See Celery. Diseases. Bacterial leaf spot.
Cercospora apii. See Celery. Diseases. Late blight.
Plasmodiophora brassicae. See Celery. Diseases. Club root.

Of course there are many other headings in the catalog besides the host names, such as BACTERIAL DISEASES OF PLANTS—PARASITISM AND DISEASE RESISTANCE—SOIL ENVIRONMENT, EFFECT ON PLANT DISEASES—MOSAIC DISEASES—SEED TREATMENT—PLANT DISEASES with many subdivisions and a geographic grouping. These few will serve as examples of a very large number.

The catalog is, of course, still in the process of making. The growth of the catalog is well indicated by the lists of current botanical literature sent out every two weeks by the Bureau of Plant Industry Library, since these lists are compilations of the indexing done for the botany catalog. These lists also serve as an indication of the present rate of botanical publication. Botanical literature has long been so voluminous as to be beyond the power of any one botanist to master or even to read. The examining and cataloging of that portion of it which reaches Washington libraries already taxes the ability of Miss Atwood and her present assistant Miss Colvin.

Some idea of the scope of the catalog may be gained from a statement of its size. Early in 1925 the author catalog contained 130,000 full entry cards. The subject catalog on general botany contained 95,000 cards and that on plant pathology 25,000 cards. This is exclusive of a large number of cross references. Comparison of the Plant Industry Catalog with other botanical catalogs is neither necessary nor desirable. To paraphrase the recently quoted statement of a wise dean regarding his college, we do not claim that it is the best catalog of the literature of plant pathology or even that it is better than others, but we know that it is a good catalog and that it is continually becoming better.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

PHYTOPATHOLOGICAL NOTES

Conference for the study of potato virus disease, Lincoln, Nebraska, December 28, 1925. The Nebraska Agricultural Experiment Station extends an invitation to every one interested in potato virus diseases for a one-day session at Lincoln preceding the American Association meetings. The objects of the conference are: (1) to study the symptoms of virus diseases and combinations of these diseases on different varieties growing in the greenhouse; (2) to arrive at a standard method of describing symptoms of virus diseases; (3) to avoid the duplication of names for the same disease; (4) to acquaint all workers with virus diseases which are already described by other workers but do not occur in their own localities; (5) to discuss the relation of virus diseases to potato seed production. Different investigators will provide plants with typical symptoms of virus diseases and will discuss and compare the symptoms as they appear in the greenhouse with those which they have observed in the field. This should enable every one attending the conference to become more familiar with the symptoms of various virus diseases, to obtain a better comparison of their destructiveness, and to formulate ideas leading to a more uniform system of potato certification.

The International Committee of Phytopathology and Economic Entomology, appointed by the first congress of Phytopathology and Economic Entomology, held in the Netherlands in 1923, has issued a report covering the period of June 30, 1923, to January 1, 1925. Dr. H. M. Quanjer, Wageningen, Holland, is chairman of the committee.

Personals.—Dr. C. H. Kauffman of the University of Michigan will be on sabbatical leave during the first semester of the academic year 1925–26. He will spend the time in the Pacific Coast states. Correspondence will be forwarded from his usual Ann Arbor address.

REPORT OF THE NINTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The ninth annual meeting of the Pacific Division of the American Phytopathological Society was held at Oregon Agricultural College, Corvallis, June 22–24, 1925, in conjunction with the Northwest Association of Horticulturists, Entomologists and Plant Pathologists. The official summer field meeting of the American Phytopathological Society also was scheduled to meet jointly with the Pacific Division at Corvallis.

A total of twenty-one plant pathological papers was presented at these meetings, most of them before the pathological section, but a few at the joint sessions of the two societies. About twenty plant pathologists representing California, Idaho, Oregon, British Columbia and the U. S. Department of Agriculture attended the meetings. There were approximately one hundred plant pathologists, entomologists and horticulturists in attendance at the joint meetings.

The program closed Wednesday afternoon, June 24, with field trips to the plots where investigations are being conducted on the virus diseases of potatoes and of the bramble fruits.

The 1926 meeting of the Pacific Division will be held at Mills College, Oakland, California, in affiliation with the Pacific Division of the American Association for the Advancement of Science. The date for this meeting has not yet been definitely set, but presumably it will be in late June.

Following is the treasurer's report as of June 30, 1925.

Receipts:		
Advanced from C. W. Hungerford	$65.52	
Dues received to June 30, 1925	16.00	
Total receipts	81.52	
Disbursements:		
Expressage	1.11	
Affiliation fee with Pacific Division, A. A. A. S.	5.00	
Stationery, clerical services and stamps	12.12	
Printing programs	8.00	
Total disbursements	26.23	
Balance		$55.29

C. E. Owens,
Secretary-Treasurer.

TITLES AND ABSTRACTS OF PAPERS READ AT THE NINTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, CORVALLIS, OREGON, JUNE 22–24, 1925

Newer facts concerning the Verticillium wilt of potatoes. M. B. McKay.

Some problems connected with seed treatment of cereals. H. P. Barss.

Resistance to western yellow blight of tomato. C. W. Hungerford.

Progress report upon potato virus disease investigations. C. W. Hungerford and J. M. Raeder.

The virus diseases of potatoes in Oregon. M. B. McKay.

Transmission of mosaics from potato to tomato. M. B. McKay.

A Myxomycete occurring in the smaller roots of beets. T. E. Rawlins.

A heavy infection by a Myxomycete has been consistently observed in the cortical cells of the smaller roots of sugar beets grown in the greenhouse. The most common form of the organism is the multi-nucleate plasmodial stage which often fills the invaded cells. The plasmodium later becomes differentiated into a mass of thick-walled hexagonal spores which presumably liberate zoospores after the death and decay of the host. An Olpidium-like form and a thick-walled resting stage have also been occasionally found in the infected roots, but it has not been determined whether they are stages in the life cycle of the organism. These preliminary studies indicate that the organism is the same as was described by Nemec as *Sorolpidium betae* Nemec. Future studies are planned to determine the degree of infection in beets grown in the field and to determine whether the organism is detrimental to the growth of the host.

A mycorrhizal fungus found in the smaller roots of celery. T. E. Rawlins and E. H. Smith.

A study of the smaller roots of celery plants grown in the delta peat soil of California has shown that they are heavily infected by a mycorrhizal fungus. The fungus is a Phycomycete and is simliar to the mycorrhizal fungus described by F. R. Jones as having been found in legumes and other plants, in that it is confined almost entirely to the cells of the inner cortex. However, it differs from the fungus found in the pea in that the intra-cellular absorbing mycelium consists of a mass of small distinct hyphae, rather than of swollen amorphous haustoria. This difference may, of course, be due to the different host and is insufficient evidence for considering it as a distinct species. Experiments are in progress in an attempt to determine the relationship existing between the celery plant and the fungus. The organism found by Jones has been observed in great abundance in the roots of sweet peas in California.

Tip-burn and "Slime" diseases of lettuce in California. T. E. Rawlins and R. L. McClain.

Lettuce was grown in both adobe and composted greenhouse soils maintained at different moisture contents. It was found that the plants tip-burned earlier and more severely in the soils of higher moisture content. Plants grown in solution culture also tip-burned very severely. These experiments indicate that it is not feasible to control tip-burn by increasing the water supply at the surfaces of the roots. Cytological studies of tip-burn in its earliest stages show that the first degeneration usually occurs in the cells adjacent to the vessels at the junction of small marginal veins. This indicates a close relationship between the transpiration stream and the degeneration. Numerous fertilizer experiments failed to show any appreciable difference in the development of tip-burn in the different plots. In general, conditions which promote succulence in the plants seem to make them more susceptible to tip-burn. The tip-burned tissues are often invaded by *Botrytis* sp. or by bacteria. These secondary invasions produce very destructive rots, locally known as "Slime."

A rot of the Calimyrna fig in California. P. D. CALDIS.

A rot of the fruit of the Calimyrna variety of figs was observed in 1922 for the first time, and is known under the names soft rot, pink rot, brown rot, stem end or eye end rot. It is also confused with souring. The rot is caused by a form of *Fusarium moniliforme* Sheldon, and occurs only in caprified figs and caprifigs. The spores of the fungus are carried into the fig at caprification time by the caprifying insect *Blastophaga psenes* L. The fungus grows on the bodies of the dead insects and the stigmas of the flowers in the cavity of the fig until ripening time, when it is able to invade the tissues of the fruit. The rot is mostly internal, but if climatic conditions are favorable, water-soaked purple or pink spots may appear on the surface. A red and a white bacterium are constantly associated with the fungus but are not the primary causal agents.

Another apple-tree anthracnose in the Pacific Northwest and a comparison with the well-known apple-tree anthracnose. S. M. ZELLER and LEROY CHILDS.

A perennial canker of apple trees is prevalent in the Pacific Northwest having a known distribution throughout some of the fruit growing districts of British Columbia, Oregon and Washington. This disease has been observed, or specimens of it received, from the Hood River and Willamette Valleys of Oregon, the Wenatchee and Spokane districts of Washington, and the Okanagan district of British Columbia. The perennial character of the canker complicates the control and is apparent in the concentric growth areas which do not occur in apple-tree anthracnose. The acervuli discharge ellipsoid conidia during spring months while the curved conidia of anthracnose are discharged during fall and winter months. The causal organism is a *Gloeosporium*, with morphological affinities close to those of the imperfect stage of *Glomerella cingulata* (Stonem.) S. and V. S. A characteristic ripe rot is produced on apple fruit in storage or by inoculation.

Spur blight (Mycosphaerella rubina) of raspberry in Oregon. S. M. ZELLER and ROBT. K. NORRIS.

This disease is widely distributed west of the Cascade Mountains wherever species of *Rubus* grow naturally or under cultivation, providing climatic conditions are favorable. In commercial plantings of western Oregon spur blight occurs on red raspberry (Cuthbert) and loganberry and with less detrimental effects on black raspberry. The greatest apparent damage is to the Cuthbert under the climatic conditions of the Ashland district, where losses at times reach 50 per cent of the normal crop. Bordeaux mixture applied when the new canes are approximately eight inches and again when they are fifteen to thirty inches has given satisfactory control with much more vigorous growth of canes.

Observations on the growth of Armillaria mellea Vahl in culture. **HAROLD E. THOMAS.**

Experiments of a preliminary nature with pieces of live roots and twigs of various trees, kept moist in culture tubes and inoculated with *Armillaria* with the object of testing them for resistance, gave the following results:

Almond roots and twigs—readily invaded by fungus, growth vigorous.

French pear twigs, roots and twigs of unidentified *Prunus* sp. found growing in "Armillaria spot"—invaded in part, growth poor.

Myrobalan roots, Cal. North. black walnut roots—not invaded.

The results show that the known resistant varieties show resistance by this method. With improved means of sterilizing surfaces the method may prove effective.

Growth of the fungus has been tested on autoclaved material with the following results:

1. Excellent growth in: autoclaved tube cultures of almond, apricot, pear and Cal. Nor. black walnut twigs; also in flask cultures of dried oak leaves, oak twigs, and dried grass, the material being ground and saturated with water before autoclaving.
2. Poor and slow growth in: flask cultures of coast live oak roots (*Quercus agrifolia* Neé) and of blue gum bark; sawdust cultures of valley oak (*Qucrcus lobata* Neé); mixtures of equal volumes of sand and valley oak sawdust; peat soil.
3. Very poor growth in: sawdust cultures of Douglas fir; cultures of equal volumes of sand and fir sawdust, soil and fir sawdust, and soil and valley oak sawdust.
4. Practically no growth in ordinary greenhouse potting soil.
5. No growth in sphagnum moss.

The green muscardine fungus (Oospora destructor (Metschn.) Delacroix) on European earwig and other insects in Oregon. H. P. **BARSS** and H. C. **STEARNS.**

A fungous disease apparently identical with the green muscardine of Europe has been collected repeatedly at Portland, Oregon, on European earwig and other insects both outdoors and in the insectary of the State Board of Horticulture. The morphological characteristics of the fungus correspond with those given by Delacroix and Vast for the European fungus *Oospora destructor.* It has been readily brought into pure culture. Artificial infections of healthy earwigs with these cultures were successful and reisolations have been made from the insects thus killed. Abundant moisture seems to be necessary to bring about infections and there seems to be little reason to expect the fungus to assume any very important role in the biological control of the earwig under the climatic conditions prevailing in the Pacific Northwest. The fungus produces a white mycelial outgrowth from between the segments of the insects. As the conidia form the color changes to a glaucous green.

A Tubercularia canker of Chinese elm. C. E. **OWENS.**

A canker disease of the Chinese elm (*Ulmus pumila* L.) was brought to the writer's attention in May, 1925, by Mr. J. L. Mielke, a forestry student, who found the disease developing on a small planting of these elms in the nursery maintained by the school of forestry on the campus of Oregon Agricultural College. An examination showed several trees completely girdled and in a dying condition. The conidial stage of a fungus was fruiting abundantly on the surface of the cankers. This proved to be a species of Tubercularia which, microscopically, seems to be identical with the imperfect stage of *Nectria cinnabarina.* Only the imperfect stage of the fungus has thus far been observed. In all cases examined the fungus seems to have entered at pruning wounds, but once in it acts as a virulent parasite, soon girdling and killing trees which otherwise seem perfectly healthy and vigorous.

Center Rot of "French Endive" or Wilt of Chicory (Cichorium intybus L.) D. B. SWINGLE.

There is a marked increase in the production of French Endive, which in the past has been extensively imported from Europe. Many places in the northern United States are well suited to the production of this vegetable, which is in strong demand during the winter season.

Difficulty has been experienced with endive rots which are under investigation at the Montana Agricultural Experiment Station. These are found to be of two types: "Center Rot" affecting chiefly the young inner leaves, and superficial rots beginning usually on the older leaves. In cultures from leaves affected with the center rot, two species of organisms have been repeatedly found. On re-inoculation each of these is capable, independently of the other, of producing the regular symptoms of center rot, which is characterized by a yellowish-olive color. Both failed to attack beets, carrots, cauliflower, cucumbers, potato and turnips, and gave doubtful, probably negative, results on head lettuce and celery. These are described and named as two species, *Phytomonas cichori* and *Phytomonas intybi.* Both are non-spore-forming rods with polar flagella, gram negative, indol negative, not hydrolyzing starch, with a thermal death point of 51°. *Phytomonas intybi* liquifies gelatin and reduces nitrates with nitrogen gas, while *Phytomonas cichori* does not liquify gelatin or reduce nitrates.

An obscure new disease of the strawberry in California. A. G. PLAKIDAS.

The productiveness of the strawberry plantations of the Central California coastal region, and especially of the Watsonville district, has been on the decline for a number of years. This decrease has been attributed by the growers to various causes, but chiefly to degeneration or "running out" of varieties. The first pathological description of a specific disease, known locally as "Strawberry Blight" was given by Prof. W. T. Horne in 1922. (Rept. of the College of Agr. and the Agr. Exp. Sta., University of California, 1922, pp. 121–123). This disease is now widely spread in the Santa Clara and Pajaro valleys, and has been found in other sections of the state—Fresno, San Joacquin, Monterey, Sonoma, and Shasta counties. The most conspicuous symptoms of the disease are: yellowing of the leaves on the margin and between the large veins, with a characteristic curling of the leaves; dwarfing of the leaves, and consequently of the entire plant; the roots of the affected plants apparently remain healthy. The affected plants usually do not die (except in rare instances where other unfavorable factors may enter in), but they remain permanently stunted, and all runners (stolons) arising from a diseased plant are, and continue to remain, diseased. The disease does not seem to be due to malnutrition, for it occurs on all kinds of soil and under the best cultural conditions. Affected vines, transplanted to rich, well manured soil did not recover. The disease apparently is not transmissible through the seed, for seedlings obtained from seed from diseased plants are healthy. No organism has been found so far to be associated with the disease. The infection spreads very rapidly. The disease has many of the characteristics of mosaic or degeneration troubles. Some preliminary experiments, carried out by Professor W. T. Horne, in an attempt to transmit the disease by inarching leaves and runners between healthy seedlings and diseased plants gave inconclusive results. It is believed that the infectious principle is transmitted from diseased to healthy plants by insects. The red spider (*Tetranychus telarius* Linn.) and the strawberry aphis (*Myzus fragaefolii*) are strongly suspected as being the carriers. Experiments to determine this are now in progress. Photographs and exsiccatae illustrating the disease were shown.

An unusual vascular browning of potato tubers as a result of frost. J. W. EASTHAM.

As a result of a severe frost which cut the tops, the last week in September, Burbank potatoes in the Courtenay District showed a browning in the vascular system accompanied by phloem necrosis. This necrosis unlike typical frost necrosis was confined with great uniformity to the vascular "rings" superficially resembling wilt rather than frost necrosis. Field observations also suggest that a succession of temperatures verging on freezing but not low enough to actually cut the tops may bring about net necrosis of the tubers in the Burbank variety. A case of "black heart" was also observed in potatoes which had been exposed to freezing temperatures. About 12 per cent of the tubers were so badly frozen that they collapsed. After sorting and sacking, the remainder were stored with good ventilation and not exposed to heating. About 8 per cent developed well marked "black heart." Potatoes of the same variety not exposed to freezing temperatures stored under identical conditions did not develop "black heart."

The control of core break-down in pears. HENRY HARTMAN.

Core break-down is essentially a disease affecting the core area of pears. It is generally a storage trouble, making its appearance as the fruit approaches prime eating condition. Thus far, it has not been possible to attribute core break-down to the work of a living organism. Although the disease is more serious in some localities than in others, it is more or less common to all pear regions. The development of core break-down is undoubtedly closely associated with time of picking. In all the tests and observations made, only the fruit harvested after its best picking time became affected. The amount of disease that ultimately develops does not seem to be influenced by either the kind of storage or by the length of the storage period. The pressure tester already described in the literature of the Oregon Station (Ore. Sta. Bul. 186 and 206) again proved to be a reliable indicator of maturity in pears, and at this time appears to be the most effective weapon in the control of core break-down. This disease has generally been controlled and pears have developed good quality when picking has been done within the following ranges of pressure: Bartlett, 35 to 25 pounds; Anjou, 24 to 19 pounds; Comice, 20 to 18 pounds; Winter Nelis, 28 to 24 pounds; Willamette Valley Bosc, 30 to 27 pounds, Rogue River Bosc, 24 to 20 pounds.

A bean disease caused by the virus of sugar beet curly-top. EUBANKS CARSNER.

A severe epidemic disease occurred in the bean crop of Twin Falls County, Idaho, in the spring of 1924. A description of the symptoms by a lay observer indicated that the disease was of the mosaic type. The time and locality in which the epidemic occurred suggested the possibility that the leafhopper, *Eutettix tenella,* which transmits the virus of the curly-top disease to sugar beets, might be responsible for the bean disease. Seed were obtained of the important bean varieties grown in southern Idaho and two of these varieties were grown in the greenhouse and inoculated with curly-top virus. The bean plants on which viruliferous leafhoppers were caged developed marked symptoms of the disease—general dwarfing and leaf distortion. The plants on which nonviruliferous leafhoppers were caged continued healthy. The virus was recovered from the diseased beans and effectively transmitted to healthy sugar beets by means of nonviruliferous leafhoppers. The greenhouse inoculations indicated a difference in susceptibility to the disease between the two bean varieties. Field inoculation trials are now in progress with seven varieties of beans in the effort to get further evidence as to variation in susceptibility or resistance. Further investigation will be necessary to definitely determine whether or not the bean disease in southern Idaho is caused by the curly-top virus.

Preliminary reports on transmission of dwarf of loganberry. S. M. ZELLER.

Aphids originally obtained from *Rosa rubiginosa* L. were allowed to feed for several days on succulently growing loganberry plants affected with the dwarf disease (PHYTOPATHOLOGY **15**: 125. 1925) and were then transferred to the young leaves of healthy loganberry plants grown under cages. Slight or severe necrosis along the veins and margins of the leaves resulted within six days and all growth since this necrosis appeared has exhibited the symptoms of dwarf which are usually observed in the field. When aphids from the same source were transferred from healthy loganberry plants to healthy loganberry plants, the latter have had no symptoms of the dwarf disease

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THE RELATION OF CERTAIN SPECIES OF PHYSALIS TO THE OVERWINTERING OF THE MOSAIC DISEASE OF CUCUMBER

M. N. WALKER[1]

WITH PLATES XXXI AND XXXII

INTRODUCTION

A knowledge of the means by which the virus of mosaic diseases of cultivated annual plants overwinters is essential in developing control measures. It is known, in the case of the cucumber and the tomato, that certain perennial plants carry the mosaic disease over winter, and that insects may transmit the disease from these plants to the cultivated host in the spring. In the case of cucumber mosaic, a number of plants have been found to act as sources of primary infection, particularly the wild cucumber, *Micrampelis lobata,* (Michx.) Greene (8, 10), the milkweed, *Asclepias syriaca* L., and the pokeweed, *Phytolacca decandra* L. The wild cucumber is an annual, but it has been shown that the mosaic disease of this plant is carried over winter in the seed. The other two plants are perennials. The present paper deals with studies of the overwintering of cucumber mosaic on perennial species of *Physalis.*

For several years, experiments on the control of cucumber mosaic have been in progress at Madison and Rockland, Wisconsin, and Marengo, Illinois, under the direction of Dr. S. P. Doolittle. During these experiments, all the known and suspected wild hosts of the disease were removed around the experimental cucumber fields in an attempt to control the disease by eliminating the sources of primary infection. While assisting in this eradication work at Rockland, Wisconsin, during the summer of 1922, the writer noticed that numbers of mosaic ground cherry plants, *Physalis pubescens*

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[1] The writer is indebted to Prof. L. R. Jones, of the Department of Plant Pathology of the University of Wisconsin, and to Dr. S. P. Doolittle, of the Office of Cotton, Truck, and Forage Crop Disease Investigations, for advice and suggestions during the progress of this work.

L. (Pl. XXXI, B), occurred near four of the most severely diseased cucumber fields. In view of the earlier work on the overwintering hosts of cucumber mosaic, it was suspected that certain species of *Physalis* might also be concerned in the overwintering of the disease, and a study was therefore made of the mosaic found on the cultivated ground cherry.

REVIEW OF LITERATURE

The first mention of mosaic on *Physalis* spp. was made in 1914 by Clinton (5), who infected two out of seven plants of a wild species of *Physalis* with expressed sap of mosaic tobacco plants. Clinton failed to mention the specific name of the *Physalis* used or to state whether it was an annual or a perennial.

Allard (1), in 1914, obtained infection on two garden species of *Physalis,* which he does not specifically name, with material from mosaic tobacco plants.

Nishimura (14), in 1917, found *P. alkekengi* L. to be a carrier of tobacco mosaic, although the plants showed no symptoms of the disease.

Crawford (6), in 1921, mentions in an abstract the fact that *Physalis longifolia* Nutt. is an overwintering agent of tomato mosaic.

Gardner and Kendrick (11, 12, 13), 1921 and 1922, who have done much work on species of *Physalis* in relation to the mosaic disease of tomato, have noted the occurrence of a mosaic disease on both annual and perennial species of *Physalis* in the neighborhood of tomato fields, as well as on several other solanaceous weeds; and they have proved the importance of the species *P. subglabrata* and *P. heterophylla* in connection with the overwintering and dissemination of tomato mosaic.

EXPERIMENTS WITH PHYSALIS PUBESCENS

Cross inoculations from Physalis pubescens to cucumber. The finding of mosaic plants of *P. pubescens* L. in the vicinity of badly infected cucumber fields near Rockland, Wisconsin, in the summer of 1922, led the writer to take specimens of the diseased ground cherry plants to Madison, where inoculations were made from them to healthy cucumbers. Crushed leaf tissue of mosaic plants was inserted in healthy plants by means of a sterile scalpel. These inoculations produced no infection, but inasmuch as the method used had proved less satisfactory than inoculation by means of aphids, in work with other mosaic diseases, it was planned to continue the work by use of these insects. Upon the next trip to Rockland, young ground cherry plants, both healthy and diseased, were dug up, taken to Madison, Wisconsin, and transplanted in the greenhouse. Aphids (*Aphis gossypii*) from a stock kept in the greenhouse on healthy cucumbers under cheesecloth cages were colonized on the mosaic *Physalis* plants, and after twenty-four hours were transferred to healthy young cucumbers under similar cages. Inoculations made in this manner produced infection in a high percentage of cases, as shown in table 1. Later, infection was obtained in a few inoculations made by the artificial method, but the percentage of infection was lower than when aphids were used. (See table 2.)

During these inoculation experiments it was found that the incubation period of the mosaic disease of *P. pubescens* ranged from five to fourteen days. Infection occurring more than fourteen days after inoculation was considered as accidental, unless doubtful symptoms had appeared before this time and later had become more definite.

TABLE 1.—*Results of inoculations of cucumber plants by means of aphids from mosaic and healthy plants of Physalis pubescens*

Date inoculated	Source of aphids	No. aphids per plant	No. plants inoculated	No. plants diseased	Date observed
	P. pubescens				
Aug. 22, 1922	Mosaic	15	12	6	Aug. 30, 1922
Aug. 22, 1922	Healthy (Control)	15	12	0	Aug. 30, 1922
Aug. 25, 1922	Mosaic	15	8	8	Aug. 30, 1922
Aug. 25, 1922	Healthy (Control)	15	8	0	Aug. 30, 1922
Mar. 31, 1923	Mosaic	20	8	7	April 5, 1923
Mar. 31, 1923	Healthy (Control)	20	8	0	April 5, 1923
April 4, 1923	Mosaic	20	10	6	April 12, 1923
April 4, 1923	Healthy (Control)	20	6	0	April 12, 1923
April 20, 1923	Mosaic	11	1	1	April 28, 1923
April 23, 1923	Healthy (Control)	15	1	1	May 1, 1923
Jan. 18, 1924	Mosaic	12	8	3	Feb. 1, 1924
Jan. 18, 1924	Healthy (Control)	12	4	0	Feb. 1, 1924
Jan. 22, 1924	Mosaic	30	15	5	Feb. 4, 1924
Aug. 5, 1924	Mosaic	20	10	5	Aug. 11, 1924
Aug. 5, 1924	Healthy (Control)	20	10	0	Aug. 11, 1924

Cross-inoculations from cucumber to Physalis pubescens. During the winter, the mosaic *Physalis* plants, which were at first thought to be perennials, died, and inoculations were made from mosaic cucumbers to healthy ground cherry plants. The results of these inoculations, set forth in tables

TABLE 2.—*Results of artificial inoculations of healthy plants of Physalis pubescens with crushed leaf tissues from mosaic and healthy plants of the same species*

Date inoculated	Source of inoculum	No. plants inoculated	No. plants diseased	Date observed
	P. pubescens			
April 21, 1923	Mosaic	8	3	May 3, 1923
April 21, 1923	Healthy (Control)	8	0	May 3, 1923
March 19, 1924	Mosaic	7	4	March 28, 1924
March 19, 1924	Healthy (Control)	7	0	March 28, 1924

3 and 4, indicate that cucumber mosaic may be transmitted to healthy ground cherry plants either by direct inoculation with aphids or by artificial inoculation. It will be noticed, however, that a much higher percentage of infection resulted when aphids were used as a means of inoculation.

TABLE 3.—*Results of artificial inoculations of healthy plants of Physalis pubescens with crushed leaf tissues from mosaic and healthy cucumber plants*

Date inoculated	Source of inoculum	No. plants inoculated	No. plants diseased	Date observed
	Cucumbers			
April 2, 1923	Mosaic	10	1	April 18, 1923
June 14, 1923	Mosaic	12	3	July 2, 1923
June 14, 1923	Healthy (Control)	12	0	July 2, 1923
Aug. 6, 1924	Mosaic	9	5	Aug. 23, 1924
Aug. 6, 1924	Healthy (Control)	9	0	Aug. 23, 1924

Cross-inoculations from pokeweed to Physalis pubescens. Pokeweed, *Phytolacca decandra* L., has been shown to have a mosaic disease identical with that of cucumber (9, 10). Aphids were colonized on mosaic pokeweeds and then transferred to healthy ground cherry plants. The results of these inoculations, given in table 5, indicate that pokeweed mosaic may be transmitted to *P. pubescens*, but the percentage of infection was lower than in the case of inoculations from the cucumber to *Physalis*. This may be accounted for in part by the fact that it is harder to colonize the cucumber aphis on pokeweeds than on the ground cherry. The symptoms produced in this case were identical with those resulting from inoculations from mosaic cucumber plants.

TABLE 4.—*Results of inoculation of healthy plants of Physalis pubescens by means of aphids from mosaic and healthy cucumber plants*

Date inoculated	Source of aphids	No. aphids	No. plants inoculated	No. plants diseased	Date observed
	Cucumbers				
March 26, 1923	Mosaic	25	8	8	April 4, 1923
March 26, 1923	Healthy (Control)	25	8	0	April 4, 1923
March 27, 1923	Mosaic	25	8	7	April 4, 1923
March 29, 1923	Healthy (Control)	25	8	0	April 4, 1923
April 8, 1924	Mosaic	25	10	4	April 17, 1924
April 8, 1924	Healthy (Control)	25	10	0	April 17, 1924
Aug. 25, 1924	Mosaic	25	12	7	Sept. 2, 1924
Aug. 6, 1924	Mosaic	25	12	11	Sept. 23, 1924

TABLE 5.—*Results of inoculations of healthy plants of Physalis pubescens by means of aphids from mosaic and healthy pokeweed plants*

Date inoculated	Source of aphids	No. aphids	No. plants	No. plants diseased	Date observed
	Pokeweed				
March 7, 1923	Mosaic	12	8	3	March 26, 1923
March 7, 1923	Healthy (Control)	12	8	0	March 26, 1923
March 21, 1923	Mosaic	20	8	1	April 4, 1923
March 21, 1923	Healthy (Control)	20	8	0	April 4, 1923
March 24, 1923	Mosaic	20	6	3	April 4, 1923
March 24, 1923	Healthy (Control)	20	6	0	April 4, 1923

Cross-inoculations from mosaic tobacco and tomato plants to Physalis pubescens. Aside from the pepper, *Capsicum annuum* L., no plant of the Solanaceae was known to be as highly susceptible to cucumber mosaic as *P. pubescens.* In order to determine whether this host was equally susceptible to tomato and tobacco mosaic, typical mosaic diseases of that family, inoculations were made from mosaic plants of these species to *Physalis.* The results of these inoculations appear in table 6 and show that *P. pubescens* is very susceptible to tomato and tobacco mosaic.

Physalis pubescens, an intermediate host by which tomato mosaic may be transmitted to cucumbers. The mosaic disease of tomato is identical

TABLE 6.—*Results of artificial inoculation of healthy plants of Physalis pubescens with crushed leaf tissues from mosaic and healthy tomato and tobacco plants*

Date inoculated	Source of inoculum	No. plants inoculated	No. plants diseased	Date observed
March 20, 1923	Mosaic tomato	8	6	April 1, 1923
March 20, 1923	Healthy tomato (Control)	8	0	April 1, 1923
April 7, 1923	Mosaic tomato	8	7	April 25, 1923
April 7, 1923	Healthy tomato (Control)	8	0	April 25, 1923
April 15, 1923	Mosaic tomato	16	15	May 2, 1923
April 7, 1923	Mosaic tobacco	8	8	April 20, 1923
April 7, 1923	Healthy tobacco (Control)	8	0	April 20, 1923
April 2, 1924	Mosaic tobacco	10	9	April 18, 1924

with that of tobacco, but it does not appear to be directly transmissible to cucumbers. To determine the relation of tomato mosaic to cucumber mosaic, both of which are transmissible to *P. pubescens,* inoculations were made from ground cherry plants, infected from mosaic tomatoes, to healthy cucumbers. This phase of the work is not extensive, but the inoculations made indicate that the ground cherry acts as an intermediate host by which tomato mosaic may be transmitted to the cucumber. In these experiments, aphids from a healthy stock kept in the greenhouse were colonized on mosaic ground cherry plants infected from mosaic tomatoes, and after twenty-four hours were transferred to healthy cucumber plants. The results of these experiments appear in table 7.

These experiments are analogous to those made by Doolittle (7) with pepper, a plant having a mosaic disease apparently identical with the

TABLE 7.—*Results of inoculation of healthy cucumbers by means of aphids from plants of Physalis pubescens infected with tomato mosaic*

Date inoculated	Source of aphids	No. aphids	No. plants inoculated	No. plants diseased	Date observed
April 20, 1923	Mosaic *P. pubescens* infected from mosaic tomatoes	20	1	1	May 1, 1923
July 13, 1924	do	25	4	2	July 20, 1924
July [illegible], 1924	do	25	2	1	July 20, 1924
Aug. 25, 1924	do	16	12	4	Sept. 2, 1924
Aug. 25, 1924	Healthy *P. pubescens* (Control)	16	6	0	Sept. 2, 1924

mosaic diseases of tomato and tobacco, and susceptible to cucumber mosaic. He found pepper to be an intermediate host through which cucumber mosaic might be transmitted to tobacco, and *vice versa.*

Symptoms of mosaic on Physalis pubescens. Although the mosaic disease manifests itself in slightly different ways on the different species of *Physalis* studied, the general characters are the same, and the mosaic symptoms on the different species vary in intensity rather than in nature. The cause appears to lie in the individual growth habits of the different species, since the symptoms vary greatly within a single species when the plants are grown during different seasons of the year, and the variations of symptoms within the genus is often no wider than in the species. The symptoms here described for *P. pubescens* are therefore essentially the same on the perennial species.

The mosaic disease first becomes manifest as a faint yellow flecking of the young leaves, an unusually decided case of which is shown in Plate XXXI, A. The mottling in this plant is of the above type, although the plant had been mosaic for some time. In other cases the symptoms consist of a general yellowing of the tip leaves accompanied by savoying and curling. This symptom, however, is always followed by a mottling of more or less striking degree. Under certain conditions, no decided symptoms can be recognized for a considerable time after inoculation, but eventually a few sharply defined, blister-like patches of deep green will appear in some of the younger leaves, although not necessarily in the youngest. There may be but one or two of these dark green areas on the leaves, but the diagnosis is reliable, for the plant will later show more pronounced mosaic characters, such as increased mottling, crinkling, or dwarfing (Pl. XXXI, B).

As the disease progresses there may be either increased or decreased mottling. When dwarfing is most pronounced there may be little mottling, but dwarfing is usually accompanied by striking malformations such as filiform and crinkled leaves (Pl. XXXI, B). In leaves of dwarfed plants the veins often stand out more distinctly than in normal plants. This apparently is due to the failure of the spaces between the veins to enlarge, leaving a much larger proportion of veins to leaf area than in healthy plants.

Under adverse growing conditions, symptoms are much less pronounced than under the most favorable conditions. Unless marked stunting and malformation has already taken place, mottling may disappear, leaving plants that are unthrifty in appearance but devoid of the usual mosaic symptoms, thus making it quite difficult, or often impossible, to diagnose the disease as mosaic. This condition is especially noticeable toward the latter part of the summer or in the greenhouse in midwinter. A faintness of symptoms, or their actual fading out, has been mentioned by other workers in connection with ground cherry as well as with other plants. Gardner and Kendrick (11) mention the fact in their work with species of *Physalis*, while Allard (4, 2) notes it on tomato, *Nicotiana glauca* and *Datura stramonium*, and Clinton (5) on the tomato. The writer (10, 15) has described a similar condition on *Phytolacca decandra* and *Nicotiana glutinosa.*

It is possible that Nishimura (14) was unable to discern symptoms on *P. alkekengi*, since his inoculations on this species were made after the first of July. The existence of carriers of mosaic diseases that do not show symptoms at some time during their growth seems doubtful. The cases of *Nicotiana glauca* (3) and *Nicotiana glutinosa* (15) may be given as examples of plants once thought to show no symptoms of tobacco mosaic though serving to carry the disease, which have since been shown to be susceptible and to show definite symptoms of mosaic.

Relation of Physalis pubescens to the overwintering of cucumber mosaic. During the winter of 1922, seed collected from mosaic ground cherry plants at Rockland, Wisconsin, was planted in the greenhouse at Madison. These trials gave no indication that the disease was seed borne. A total of 1031 seedlings was grown from the seed of mosaic plants, but none of the plants showed any evidence of mosaic. It is probable, therefore, that the cultivated annual ground cherry is not a factor in overwintering of cucumber mosaic.

EXPERIMENTS WITH PERENNIAL SPECIES OF PHYSALIS

Although the cultivated ground cherry apparently was not concerned in the overwintering of mosaic, it was known that certain perennial *Physalis* species were affected with mosaic. A study therefore was made of all the plants of this genus which could be found in the vicinity of the experimental fields at Rockland, Wisconsin. In preceding years, wild cucumbers and milkweeds were found in considerable numbers at Rockland, many of them mosaic; but, as a result of the earlier eradication of these hosts, no wild cucumber and but few milkweeds were present during 1923. The discovery of large numbers of mosaic plants of two wild perennial species of *Physalis*, *P. heterophylla* Nees (Pl. XXXII, B) and the more abundant *P. subglabrata* McKenzie and Bush (Pl. XXXII, A), indicated the possibility of their being an important factor in the overwintering of the mosaic disease of the cultivated ground cherry and the cucumber, since the annual ground cherry was known to be susceptible to cucumber mosaic. These mosaic perennials apparently were limited to the neighborhood of fields then planted to cucumbers or were on land that had been in cucumbers at some time previously. Efforts were made to transfer roots of plants of both wild species to the greenhouse, but in most cases the plants died. Seed of both species were obtained from outside sources, but it was found upon planting that the seed labelled *P.*

heterophylla produced plants annual in habit; consequently the work on this species was restricted to inoculations made from a few mosaic *P. heterophylla* plants grown from roots brought in from the field.

Cross-inoculation from perennial species of Physalis to the cucumber. Since preliminary cross inoculation work indicated that the mosaic diseases of *P. pubescens* and *P. subglabrata* were identical, it was thought unnecessary to duplicate all the work done with *P. pubescens* on *P. subglabrata.* Since both *P. heterophylla* and *P. subglabrata* were susceptible to cucumber mosaic, it seemed probable that these perennial species were sources of primary mosaic infection to cucumber in the field. To prove that this was indeed the case, inoculations from mosaic *P. subglabrata* and *P. heterophylla* were made to cucumbers by means of aphids. The results of these experiments are given in table 8. Results of the reciprocal inoculations, mosaic cucumber to *P. subglabrata,* appear in table 9. These results show that the

TABLE 8.—*Results of inoculation of healthy cucumber plants by means of aphids from mosaic and healthy species of perennial Physalis*

Date inoculated	Source of aphids	No. aphids	No. plants	No. plants diseased	Date observed
March 29, 1924	Mosaic *P. subglabrata*	20	8	3	April 8, 1924
March 29, 1924	Healthy *P. subglabrata* (Control)	20	8	0	April 8, 1924
May 15; 1924	Mosaic *P. subglabrata*	20	8	2	May 23, 1924
May 15, 1924	Healthy *P. subglabrata* (Control)	20	8	0	May 23, 1924
March 22, 1924	Mosaic *P. heterophylla*	20	6	4	March 29, 1924
March 22, 1924	Healthy *P. heterophylla* (Control)	20	6	0	March 29, 1924
May 15, 1924	Mosaic *P. heterophylla*	20	4	1	May 23, 1924
May 15, 1924	Healthy *P. heterophylla* (Control)	20	4	0	May 23, 1924
June 27, 1924	Mosaic *P. heterophylla*	20	6	1	July 7, 1924
June 27, 1924	Healthy *P. heterophylla* (Control)	20	4	0	July 7, 1924

mosaic diseases of cucumbers and perennial species of *Physalis* are intertransmissible. This fact, coupled with the ability of aphids to transmit the disease, makes the relationship of perennial wild *Physalis* species to cucumber mosaic seem an important one.

TABLE 9.—*Results of inoculation of healthy plants of Physalis subglabrata by means of aphids from mosaic and healthy cucumber plants*

Date inoculated	Source of aphids	No. aphids	No. plants	No. plants diseased	Date observed
	Cucumber				
March 26, 1924	Mosaic	20	12	2	April 23, 1924
March 26, 1924	Healthy (Control)	20	10	0	April 3, 1924
April 8, 1924	Mosaic	25	10	4	April 20, 1924
April 8, 1924	Healthy (Control)	25	5	0	April 20, 1924
May 20, 1924	Mosaic	20	10	2	May 30, 1924

Importance of perennial Physalis species in the overwintering of cucumber mosaic. The probability of perennial species of *Physalis* being an important source of primary mosaic infection led to the eradication of these plants, together with others, in the experimental area at Rockland, Wisconsin, during 1923. Wherever wild *Physalis* plants were found, they were removed, the area being inspected throughout the summer at intervals of approximately two weeks. At the end of the summer of 1923, the amount of mosaic at Rockland had been greatly reduced as compared with that of the preceding season; and, as the mosaic of milkweeds and wild cucumbers had been practically exterminated during the preceding seasons, it seems probable that the reduction in the amount of the disease was due in great part to the removal of the ground cherry, the only remaining wild host of importance. Similar results were obtained in 1924 and will be published in a forthcoming paper by Doolittle and the writer.

The results of these eradication experiments, and the fact that cross-inoculations have shown that the perennial ground cherries carry cucumber mosaic over winter, indicate that these plants are important as sources of primary infection to the cucumber in the spring. This may easily be the case, for mosaic plants of *P. subglabrata* have been observed at Rockland, Wisconsin, early in May, a month before cucumbers appear above ground. Insects, particularly aphids, may transmit the disease from these plants directly to the cucumber, or they may infect the garden species of *Physalis*, from which the cucumbers may later be infected. It is possible that cucumbers in the field are infected by both of these methods. Flea beetles are commonly present on species of *Physalis*, and preliminary work by Gardner and Kendrick (11) indicates that these insects may transmit the mosaic disease. Aphids are probably of greatest importance, however, and when mosaic once appears in a cucumber field and these insects are abundant, the disease spreads very rapidly. In the fall, cucumber plants die before the wild ground cherries, and aphids or other insects moving to these plants which are still green may transmit cucumber mosaic to them, thus establishing a source of primary infection the following spring. The number of mosaic perennial *Physalis* plants is likely to increase constantly, since they are susceptible to mosaic diseases of several types, and the disease is probably often transmitted from one wild host to another. The possible importance of this genus is increased by the fact that Gray lists eight perennial species of *Physalis*, representatives of which are widely scattered over the entire United States.

SUMMARY

1. The mosaic disease of *Physalis pubescens* L. is readily transmitted to the cucumber by means of the cucumber aphis, and cucumber mosaic is also transmissible to *P. pubescens* by the same means. Infection can be secured between these hosts by artificial inoculation also, but the percentage of infection is lower by this method.

2. The mosaic diseases of pokeweed, tobacco, and tomato are also transmissible to *P. pubescens.*

3. *Physalis pubescens* is an annual, and the disease is not transmitted through the seed.

4. It has been found that a mosaic disease lives over winter in two perennial species of *Physalis, P. subglabrata* and *P. heterophylla.* The mosaic disease on these plants is readily communicated to the cucumber by means of the cucumber aphis and by artificial inoculation, and is also transmissible to *P. pubescens.*

5. Field observations indicate that mosaic infection is common on perennial species of *Physalis* in the vicinity of cucumber fields, and the results of field experiments indicate that these wild hosts are an important source of primary infection for the cucumber.

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FORAGE CROP DISEASE INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE.

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DESCRIPTION OF PLATES

PLATE XXXI

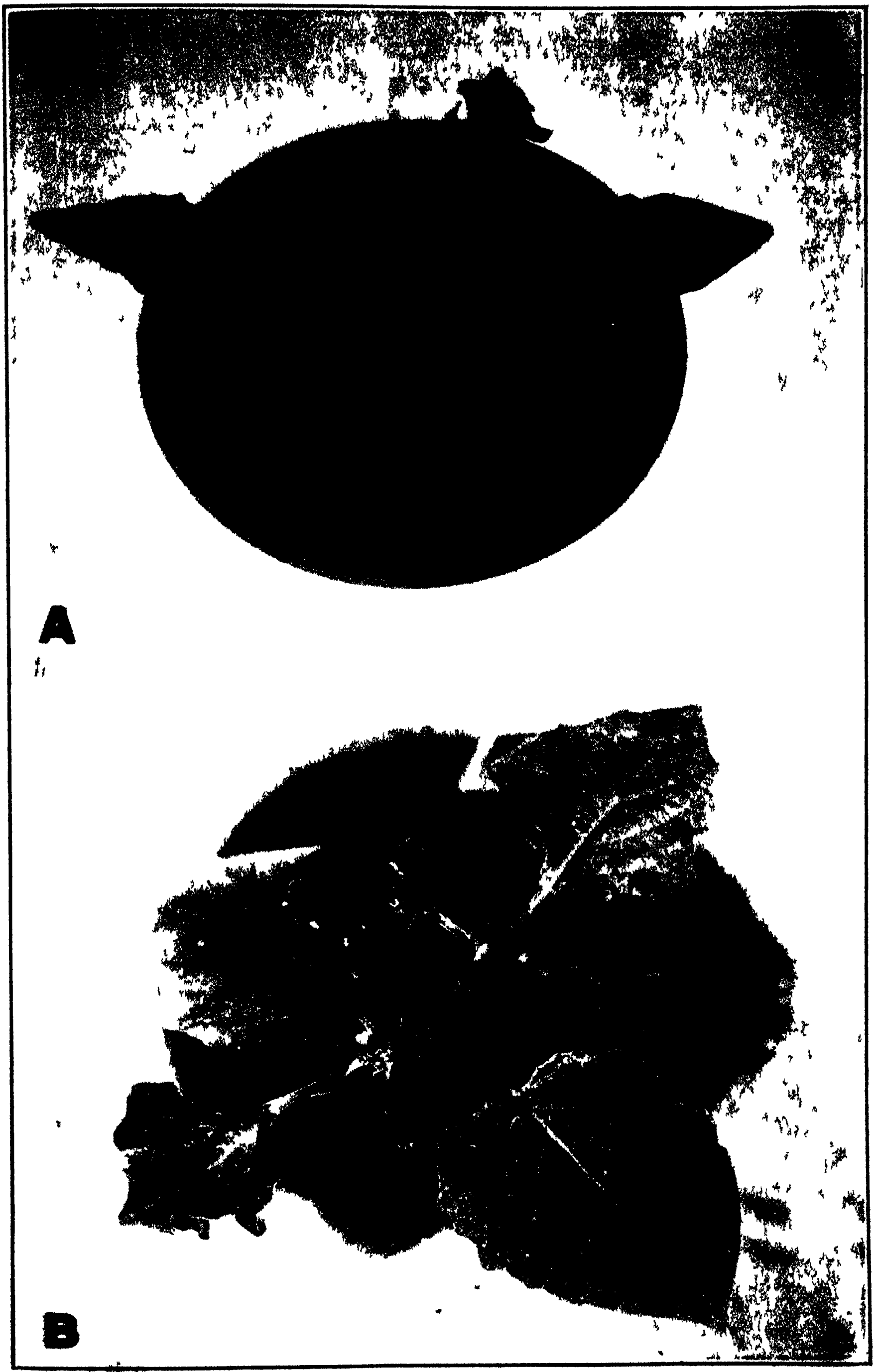
A. A mosaic plant of *Physalis pubescens* showing mottling, crinkling and dwarfing. Mottling of the type shown here is common in plants showing the first symptoms of the disease, the other symptoms appearing later.

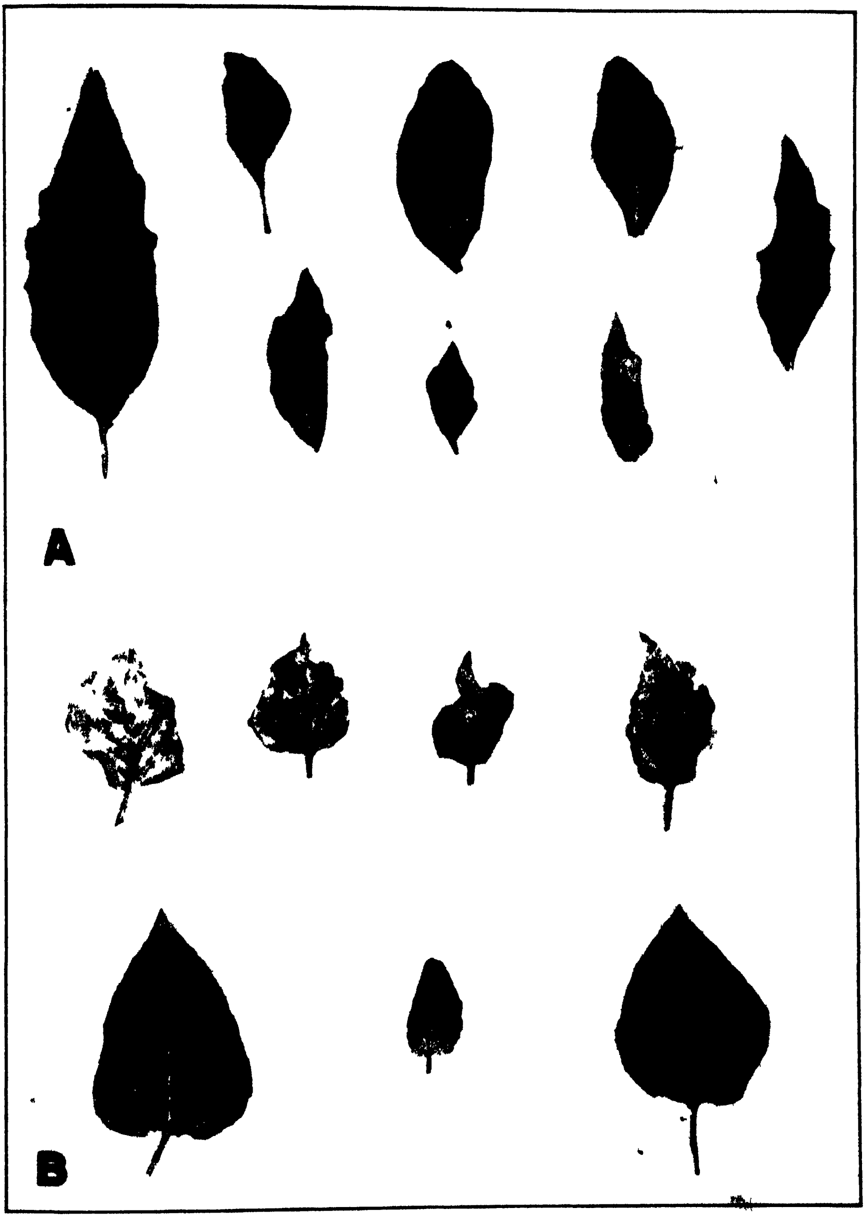
B. Mosaic plant of *P. pubescens* showing an acute degree of crinkling.

PLATE XXXII

A. Leaves of *Physalis subglabrata*; leaves at extreme right and left are healthy, leaves in center are mosaic.

B. Leaves of *Physalis heterophylla*; the upper row are mosaic, lower row healthy.


A
B


A
B

ATTENUATION OF THE VIRUS OF SUGAR BEET CURLY-TOP

EUBANKS CARSNER[1]

WITH PLATES XXXIII TO XXXVII

STUDIES WITH CHENOPODIUM MURALE

The fact has been previously reported (2) that by passage of the virus of the curly-top disease of sugar beets through nettle-leafed goosefoot, *Chenopodium murale,* the virus is so attenuated that when transmitted to healthy young sugar beets it either fails to cause the curly-top disease or usually produces only mild cases. Thirty-three experiments have been recorded in which leaf-hoppers reared on *Chenopodium murale,* progeny of viruliferous parents, have been caged on one or more healthy sugar beets or other susceptible plants. These progenies, as demonstrated earlier (4), would under these circumstances have had to acquire the virus from the *C. murale* on which they were reared in order to transmit it to other plants. In twenty of the thirty-three tests, the plants to which the leafhoppers from *C. murale* were transferred remained healthy. In thirteen of the tests, some or all of the plants developed symptoms of curly-top. Nineteen of the sugar beets used in these thirteen tests developed the first symptom of the disease, the clearing of the small veins of the youngest leaves; and of these nineteen, fifteen subsequently lost all symptoms of the disease or developed only very mild cases, manifesting little or no dwarfing and only inconspicuous vein swellings.[2] In some of the tests, chickweed, *Stellaria media,* was used in addition to beet plants to test the leafhoppers from *C. murale.* Chickweed is very susceptible to curly-top infection. The details of one test with it are worth noting (Table 1).

The fact that the leafhoppers may secure the attenuated virus from *Chenopodium murale* does not interfere with their securing and transmitting the more active virus. Tests have been made in which leafhoppers reared on *C. murale* produced the mild form of curly-top in beets and were then caged for a few days on a plant affected with the severe form of the

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[1] The writer wishes to acknowledge the collaboration of Mr. C. F. Stahl, formerly of the Office of Truck-Crop Insects, Bureau of Entomology, in some of the work reported, and the assistance of Mr. C. F. Lackey, junior pathologist, Office of Sugar-Plant Investigations, Bureau of Plant Industry, in some of the experiments.

[2] In some of the earlier of these tests records were made of the appearance of curly-top symptoms, but no subsequent record was taken. It is not, therefore, definitely known that four of the nineteen affected beets developed mild cases of curly-top.

TABLE 1.—*The attenuation of curly-top virus by Chenopodium murale, as indicated by inoculating Stellaria media with virus from Chenopodium murale*

Date	Results of inoculating *Stellaria media* with virus from *Chenopodium murale*	Results of inoculating *Stellaria media* with virus from a severely affected curly-top beet
Nov. 14, 1923	Seven leafhoppers reared on *C. murale* from viruliferous females were caged on *S. media*	Two leafhoppers from severely affected beet were caged on *S. media*
Nov. 19	Healthy	Diseased
Dec. 1	Diseased—faint but definite symptoms	Severely diseased
Dec. 4	No chlorosis	Marked chlorosis of terminal leaves
Dec. 17	Slight chlorosis of terminal leaves	Dead

disease and finally transferred to healthy beets. The beets to which these insects were at last transferred developed severe cases of curly-top. Beets affected with the mild form of the disease are neither rendered immune from nor more resistant to the severe form of the disease than are healthy beets. Leafhoppers from a severely affected curly-top beet have been caged on beets affected with the mild form of the disease, as well as on healthy controls. The symptoms of the severe disease developed in both lots.

STUDIES WITH RUMEX CRISPUS

Curly dock, *Rumex crispus,* is another plant which, with *Chenopodium murale,* was previously reported (1) as nonsusceptible to curly-top because nymphs reared on it from viruliferous females failed to produce curly-top when transferred to sugar beets. This result was obtained with an old dock plant which was transplanted to a pot from the field. Further study of the matter was undertaken when Dr. H. P. Severin stated to the writer that he had obtained curly-top symptoms on the dock by inoculating young plants. The later tests have been made with plants grown from seed. These tests show that *R. crispus* is resistant to the action of the virus and that the virus is attenuated by passing through it. It will make the results clearer to give the tests in detail (Tables 2 and 3).

STUDIES WITH SUAEDA MOQUINI

Another plant which is of interest in regard to attenuation of the curly-top virus is alkali blite, *Suaeda moquini* Greene. This perennial is probably

TABLE 2.—*Symptoms of curly-top produced on Rumex crispus inoculated by means of leafhoppers*

Exp. No.	Date	Treatment and results
1	Sept. 6, 1924	Two viruliferous leafhoppers caged on each of 12 *Rumex crispus* plants. Plants had 4 true leaves. Controls of same age were not inoculated. Insects removed Sept. 10.
	Sept. 17	Eleven of the 12 inoculated plants showed slight, irregular vein swellings on the youngest unfolded leaf.
	Oct. 11	The leaves of the diseased plants showed no marked evidence of dwarfing or rolling. Some of the leaves showed a few scattered, rough swellings of the veins on the lower surfaces, with distinct indentations of the upper surfaces over these swellings. The plants were evidently very mildly affected.
	Nov. 5	Definite symptoms of curly-top could be found only by very close examination of those plants which had previously shown clear-cut symptoms.
2	Sept. 3, 1924	On each of 4 young *R. crispus* plants, 6 viruliferous female leafhoppers were caged. On each of 4 similar plants, 6 nonviruliferous leafhoppers were caged.
	Nov. 5	One of the plants with viruliferous insects was definitely diseased, showing fairly conspicuous vein swellings and dwarfing.
	Nov. 18	The leaves of the plant affected on November 5 were about half as long as those of the controls. *R. crispus* was not nearly so severely affected as a beet of the same age would have been under the same conditions. The other three inoculated plants showed inconspicuous vein swellings and slight dwarfing (PLATE XXXIII). The weights in grams of the entire plants (leaves and roots) were: 4 controls, 31 to 38.5, total 142.5; 4 inoculated plants, 12 to 26, total 74.
3	Dec. 19, 1924	Seven viruliferous leafhoppers were caged on a *R. crispus* plant which had 5 true leaves. Seven nonviruliferous leafhoppers were caged on a similar plant. Insects removed after about 10 days.
	Jan. 19, 1925	A few very inconspicuous vein swellings could be found on the inoculated plant, but it was as large and as vigorous as the control.

TABLE 3.—*The attenuation of curly-top virus by Rumex crispus, as indicated by inoculating sugar beet plants with the virus from Rumex crispus*

Exp. No.	Date	Procedure and results
1	Nov. 17, 1924	From the 4 diseased *R. crispus* plants, marked A to D, of experiment 2, table 2, leafhopper nymphs were transferred to healthy sugar beets as follows: from A, 20 nymphs; from B, 15 nymphs to each of two beets; from C, 6 nymphs; from D 63 nymphs.
	Dec. 2	Beet A was too heavily loaded with insects—62 nymphs were recovered from it and transferred to a fresh healthy beet (AA).
	Dec. 3	Condition of beets: A was healthy; B′ and B″ showed conspicuous vein symptoms on the younger leaves—no evidence of dwarfing; C showed definite, conspicuous vein clearing of the three youngest leaves, but the symptoms were less advanced than in the B beets; D had not grown enough to show symptoms—discarded by mistake.
	Dec. 19	The four beets A, B′, B″, and C and beet AA all showed definite symptoms of curly-top—vein swellings—but all seemed only mildly affected.
	Jan. 21, 1925	All five of the beets had recuperated or continued as mildly affected cases. All showed vein swellings on the older leaves. Some showed faint clearing of the younger leaves, while with others the younger leaves appeared normal.
2	Nov. 6, 1924	Fifteen viruliferous adult leafhoppers were caged on a young *R. crispus* plant.
	Dec. 19	The *R. crispus* plant showed no dwarfing but did show scattered vein swellings on some of its leaves. Thirty-three nymphs were transferred from it to each of two healthy young sugar beets.
	Jan. 19, 1925	Both of the beets were healthy.
3	Dec. 23, 1924	Four leafhoppers reared on curly-top *R. crispus* (inoculation experiment No. 2) were caged on each of 20 small healthy sugar beet seedlings. On each of 20 similar seedlings 4 leafhoppers from a severely affected curly-top beet were caged.
	Dec. 31	Plants with insects from *R. crispus*: of 18 plants surviving, 7 were diseased. Plants with insects from curly-top beet: of 18 plants surviving, 18 were diseased.

TABLE 3.—(*Continued*)

Exp. No.	Date	Procedure and results
	Jan. 6, 1925	Plants with insects from *R. crispus*: of 18 plants surviving, 16 were diseased.
	Jan. 12	Contrast between the two lots of beets was beginning to give evidence of the attenuation of the virus by *R. crispus*. The first pair of true leaves of the plants infected with the virus from *R. crispus* were as curled and showed fully as conspicuous vein proliferations as did the corresponding leaves of the other lot, but were more nearly normal in length. The younger leaves (third and fourth true leaves) showed fewer conspicuous swellings on the veins and were noticeably less dwarfed than the corresponding leaves of the plants infected with the more active virus (PLATE XXXIV, A, B).
	Feb. 4	Contrast between the two lots was marked. All the plants infected with the attenuated virus were mildly and uniformly affected. Those of the other lot were severely affected (PLATE XXXIV, C, D).

of considerable importance in helping to tide the beet leafhopper over the dry periods of fall and early winter when favorable annuals are not yet available for food. Severin (3) reports collecting the insects from *Suaeda moquini* and from *S. depressa,* which closely resembles the species under consideration. The writer and his colleague, C. F. Stahl, have on several occasions collected *Eutettix tenella* from *S. moquini.* As opportunity has afforded, studies have been made of this plant as to susceptibility to curly-top and the effect of the plant on the virus. The tests thus far made may well be given in detail (Table 4).

ATTENUATION UNDER NATURAL CONDITIONS

The few experiments which have been made with *Suaeda moquini* clearly show, in the writer's opinion, that this species is resistant to the injurious effect of the curly-top virus and attenuates that virus in a way comparable with the two other species discussed. The attenuation which it causes probably indicates the explanation of the facts discovered in testing leafhoppers collected from the vicinity of Bakersfield, California. As will be explained further, a considerable proportion of these insects were found to have the curly-top virus in an attenuated condition. It is not meant that the *Suaeda* was responsible for all the attenuation which occurred, but that this attenuation was brought about by the passage of the virus through uncongenial host plants.

TABLE 4.—*Attenuation of curly-top virus by Suaeda moquini, as indicated by inoculating sugar beet plants by means of viruliferous leafhoppers from Suaeda moquini*

Exp. No.	Date	Procedure and results
1	Feb. 21, 1924	Four adult leafhoppers collected from *S. moquini* near Bakersfield, California, were caged on a healthy sugar beet seedling.
	Mar. 5	Beet was healthy. Leafhoppers having the unattenuated virus would have produced curly-top symptoms in such a beet in less time than had elapsed.
	Mar. 18	The beet showed symptoms of the mild form of curly-top.
2	Sept. 18	On a small *S. moquini* plant, three viruliferous female leafhoppers were caged.
	Oct. 28	Leafhopper nymphs had hatched out on the plant. Five of these nymphs were transferred to each of five healthy young sugar beets.
	Nov. 4	One of the five beets showed faint but definite veinlet clearing, the early symptom of curly-top.
	Nov. 7	The veinlet clearing noted on November 4 had become very inconspicuous, but faint veinlet clearing was visible on the next smaller leaf, the youngest. Apparently the beet was but mildly affected.
	Dec. 3	The diseased beet showed vein swellings—very inconspicuously—on only one leaf, a leaf now mature. The plant was not dwarfed. The other four beets were healthy.
3	Nov. 7 and Nov. 17	On November 7, five more nymphs were transferred to each of two young healthy beets from the *Suaeda* plant of test No. 2; and on November 17, 25 nymphs were transferred to another beet.
	Dec. 19	All three beets were healthy. The *Suaeda* plant (inoculated September 18) had grown vigorously, and no evidence of the disease could be found on it.
4	Nov. 6. 1924	On each of four *S. moquini* seedlings, about three inches tall, five viruliferous male leafhoppers were caged. On each of two similar plants, five nonviruliferous males were caged.

TABLE 4.—(*Continued*)

Exp. No.	Date	Procedure and results
	Dec. 15	All four inoculated plants were stunted. The controls were approximately twice as large (PLATE XXXV, C, D). Close examination revealed occasional irregular swellings on some of the leaves of two of the affected plants (PLATE XXXV, A, B). These two plants had been kept against the south wall of the greenhouse—a position better lighted and relatively warmer than that where the other two inoculated plants were kept. The two latter plants did not show the leaf swellings. The viruliferous insects were removed, and ten nonviruliferous insects were caged on each of the four inoculated plants.
	Dec. 18	The leafhoppers were transferred from each *Suaeda* plant to a separate young healthy beet.
	Jan. 6, 1925	One of the beets showed the early stage of vein clearing on a part of the youngest leaf. The other three were healthy.
	Jan. 21	Another one of the beets was noted as diseased. The age of the leaves showing symptoms indicated that the first symptoms had appeared about a week earlier. Both of the diseased beets were mildly affected. Record was not made of which *Suaeda* plants the insects came from which produced the disease. Probably they were from those two which showed the leaf swellings.

The tests with the beet leafhoppers from the Bakersfield district were made on young sugar beets in a greenhouse at Riverside, California. The insects were collected at various times in the late winter and spring of 1924 from sugar beet fields and wild vegetation. A total of 801 leafhoppers were tested either singly or in small groups. A few of the tests given in detail will suffice to show the trend of the results (Table 5).

The fact that, in the tests with the leafhoppers from the Bakersfield district, most of the insects failed to produce curly-top symptoms within periods ten to twenty days longer than is required by leafhoppers with the more effective virus, and that most of the cases of the disease which they did produce were mild cases, justifies the conclusion that a considerable proportion of these leafhoppers had the virus in an attenuated form. This deduction is supported by field observations at Bakersfield. In the first place, the small amount of disease evident on March 14 in two beet fields where leafhoppers were found present early in February was contrary to what would

TABLE 5.—*The attenuation of curly-top virus under field conditions, as indicated by inoculating sugar beet plants in the greenhouse by means of leafhoppers collected from sugar beet fields and wild vegetation*

Exp. No.	Date	Procedure and results
1	Feb. 25, 1924	Fifty-seven adult leafhoppers collected from sugar beet fields and wild vegetation were caged on healthy young sugar beets as follows: 5 on each of 4 small plants and 37 on one larger plant.
	Mar. 5	One of the plants with 5 insects showed symptoms of curly-top. The other 4 were healthy.
	Mar. 29	The diseased plant was mildly affected. The plant with 37 insects showed mild symptoms on its youngest leaf. Under the same conditions leafhoppers with the unattenuated virus would have produced the severe form of curly-top in six to ten days.
2	April 15	On the plant of test No. 1, which showed symptoms on March 5 and from which the leafhoppers from Bakersfield had been removed, 15 nonviruliferous leafhoppers were caged. Ten nonviruliferous insects were caged on a severely affected curly-top beet.
	April 23	From the mildly affected beet, 6 leafhoppers were transferred to one young sugar beet and 7 to another. From the severely affected plant, 5 insects were transferred to a young sugar beet plant and 4 to another. These latter plants were for controls.
	May 2	The plant with 7 insects showed symptoms of curly-top. The one with 6 insects was healthy. Both controls were diseased.
	Sept. 4	The plant with 6 insects had developed a mild case of curly-top. The date on which symptoms first appeared had not been recorded. Both plants were still very mildly affected.
3	March 25	The insects used were collected from sugar beet fields and from wild vegetation—*Erodium cicutarium* and *Atriplex semibaccata*. From beet fields: 162 insects were caged singly on healthy beet seedlings and 36 on a larger beet plant. From wild vegetation: 81 insects were caged in odd lots on 6 beets. As a control, one viruliferous leafhopper was caged on each of 4 beet seedlings.
	March 31	Three checks diseased. All other plants healthy.
	April 2	Plants with insects from beet fields: 6 of those with single insects and the one with 36 insects showed curly-top symptoms. Plants with insects from wild vegetation all healthy.

TABLE 5.—(*Continued*)

Exp. No.	Date	Procedure and results
	April 10	Three of 4 checks diseased. Thirty-two diseased of 162 plants on which insects from beet fields were caged. Six plants with insects from wild vegetation all healthy.
4	April 22	Seventy-five leafhoppers collected from beet fields on April 19 were caged singly on healthy young beets. Twelve viruliferous leafhoppers were caged singly on similar plants.
	May 3	One of the 75 beets was diseased. Eight of the 12 checks were diseased.
	May 10	Ten of the 75 beets were diseased. Nine of the 12 checks were diseased.
	May 28	Twenty-two of the 75 beets were diseased—all mild cases. The 9 diseased checks were all severely affected.

have been expected if the leafhoppers had had the more active virus. Of course the relatively low temperatures of the period retarded the development of the disease, but it is improbable that this one factor entirely accounted for the situation. In the second place, a considerable proportion of the plants which were diseased early in the season (April 8) were only mildly affected later in the spring (May 16). When the latter observation was made, these mildly affected plants showed prominent vein swellings on some of the older leaves while the newer leaves were nearly normal in appearance. Observations in a commercial sugar beet field at Riverside, California, indicate that in this district a considerable proportion of the numerous beet leafhoppers present had the curly-top virus in an attenuated form comparable to that found at Bakersfield. On May 29, 1924, approximately twenty-five per cent of the plants showed disease. Basing their decision on the size of the plants, the amount of disease, and the number of leafhoppers present, some sugar beet growers would have ploughed up the field. On July 14, the field presented a much better appearance than had been anticipated. Nearly all the plants in the field showed symptoms of curly-top, but a large proportion of them were merely mildly affected. The grower reported a profitable yield from the field.

Further study of the effect of the attenuated virus on the growth of sugar beets was conducted in the late summer and fall of 1924. The findings can be made clearer by giving the procedure (Table 6).

TABLE 6.—*Results of inoculating sugar beet plants with attenuated and unattenuated curly-top virus by means of leafhoppers*

Exp. No.	Date	Procedure and results	
		With attenuated virus	With unattenuated virus
1	July 3, 1924	Twelve nonviruliferous leafhoppers caged on a mildly diseased beet which was inoculated by a leafhopper from the Bakersfield district on April 22.	Twelve nonviruliferous leafhoppers caged on a severely diseased beet—one of the controls of the test of April 22.
	August 12	Fifteen leafhoppers, adults and nymphs, from the mildly affected beet were caged singly on small healthy beet seedlings.	Ten leafhoppers, mostly nymphs, were caged singly on similar beets which were growing under the same conditions.
	August 18	Two of the fifteen were diseased.	All ten were diseased.
	August 23	Twelve of the fifteen were diseased.	All ten were severely affected.
	Sept. 13	Two plants remained healthy. Two which once showed symptoms now appeared entirely normal. Ten of the mildly affected plants weighed 307.5 grams (PLATE XXXVI, B).	One of the check plants was dead. Nine check plants weighed 12.4 grams (PLATE XXXVI, A).
2	Sept. 23	Fifteen adult leafhoppers which had been reared on the mildly affected beet in experiment 1 were caged singly on young healthy beets. Similar plants were left uninoculated under similar conditions.	Fifteen adult leafhoppers from a severely affected curly-top beet were caged singly on young healthy beets.
	Oct. 9	Eleven diseased — all mild cases.	Twelve diseased—eleven severely affected.
	Nov. 11	The diseased plants were dwarfed in comparison with the plants uninoculated but were less affected than the diseased checks (PLATE XXXVII). Ten of the mildly affected plants weighed 155 grams. Fourteen healthy plants weighed 625 grams.	Twelve of the fifteen were severely and uniformly affected and weighed 58 grams.

DISCUSSION

Inasmuch as the attenuation of a virus causing plant disease has not previously been reported, so far as the writer is aware, and because the developments at different stages in the experiments are significant, the details of the principal tests which demonstrated the attenuation have been given.

In view of the evidence that the virus which produces the mild disease is the same as that which causes severe curly-top, except for the fact that it is attenuated, and the essential similarity of symptoms in the two cases, the writer deems it advisable to consider the mild disease as a mild form of curly-top rather than as a distinct virus disease.

The question may arise as to why there are such regional variations in curly-top epidemics as have been observed between central and southern California. Epidemics of the severe form of the disease have repeatedly occurred in the central part of the state, while in the southern part the mild form of the disease has been the dominant type—at least during the past two seasons. There is no marked difference in the climate of the two districts and the floras are similar. A difference between the two regions, which may be significant, is that sugar beets have been grown continuously and extensively for many years in central California, while in the area under consideration in the southern part of the state the crop has been grown only intermittently and in relatively small acreages. In view of these facts, it may be that the virulence of the virus has been maintained in the one case because beets were available each season, while, in the other, attenuation resulted because the virus was carried over mainly in wild plants which caused attenuation. Attenuation unquestionably occurs in regions subject to epidemics of the severe disease, but is usually unnoticed because the severe form of the disease predominates and even masks the mild form. It is easy to understand how the unattenuated virus may be overwintered in an area where it is abundant because, in addition to the attenuating plants, there are other agencies which are known to carry the virus over winter and which do not attenuate it. These are: diseased beets from the previous season, susceptible (non-attenuating) wild annuals, such as *Erodium cicutarium,* and the viruliferous beet-leafhoppers themselves.

The question as to whether the attenuated virus may again become virulent is still open. The evidence which thus far has been obtained is not conclusive. It may be said, however, that, in the writer's tests, virulence has not been restored by quickly passing the attenuated virus through sugar beets or even more susceptible plants.

SUMMARY

The three species, *Chenopodium murale, Rumex crispus,* and *Suaeda moquini,* which have been found very resistant to the virus of curly-top, have been studied in regard to their effect on the virus. It has been found that, on passage through these plants, the virus becomes so attenuated that it causes merely a mild form of curly-top when transferred to healthy beets or other susceptible plants.

The attenuated virus has been found to occur in beet-leafhoppers when collected from their natural breeding areas, and it has been assumed that the attenuation in these cases had resulted from the passage of the virus through resistant plants, of which the three species before mentioned are probably only representatives.

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EXPLANATION OF PLATES

PLATE XXXIII

A. A leaf of a *Rumex crispus* plant showing curly-top symptoms. Note the inconspicuous swellings on the veinlets. On September 3, 1924, six viruliferous leafhoppers were caged on the plant from which this leaf was taken. Photographed November 18, 1924. (See Table 2, Exp. 2.)

B. Healthy *R. crispus* leaf from a plant on which six nonviruliferous leafhoppers were caged as a check. (See Table 2, Exp. 2.)

PLATE XXXIV

A. Sugar beets on each of which four leafhoppers from a severely affected curly-top beet were caged on December 23, 1924. Photographed January 12, 1925. (See Table 3, Exp. 3.)

B. Sugar beets on each of which four leafhoppers which had obtained the curly-top virus from diseased *R. crispus* were caged December 23, 1924. Photographed January 12, 1925. Note that the younger leaves show conspicuous symptoms of curly-top but that they are not so dwarfed as are those in A.

C. The same pot of plants shown in A. Photographed February 4, 1925. Eighteen of the 20 plants inoculated survived; all were severely affected.

D. The same pot of plants shown in B. Photographed February 4, 1925. Eighteen of the 20 plants inoculated survived; 16 were mildly affected.

PLATE XXXV

A. Tip of main branch of a healthy *Suaeda moquini.* On November 6, 1924, five nonviruliferous leafhoppers were caged on the plant shown in C, from which this tip was taken. Photographed December 15, 1924. × 2 approx. (See Table 4, Exp. 4.)

B. Tip of main branch of diseased *S. moquini.* Note papillae on leaves. On November 6, 1924, five viruliferous leafhoppers were caged on the plant shown in D, from which this tip was taken. Photographed December 15, 1924. × 2 approx.

C. Control *S. moquini.* Five nonviruliferous leafhoppers were caged on this plant on November 6, 1924. Photographed December 15, 1924.

D. Curly-top of *S. moquini.* Five viruliferous leafhoppers were caged on this plant on November 6, 1924. Photographed December 15, 1924. Note size in comparison with healthy control. From *Suaeda* plants thus affected, the attenuated virus has been transferred to sugar beets, producing curly-top symptoms.

PLATE XXXVI

A. Sugar beets with severe form of curly-top. On August 12, 1924, a leafhopper from a severely affected curly-top beet was caged on each of these nine plants. Photographed September 13, 1924. Same magnification as B.

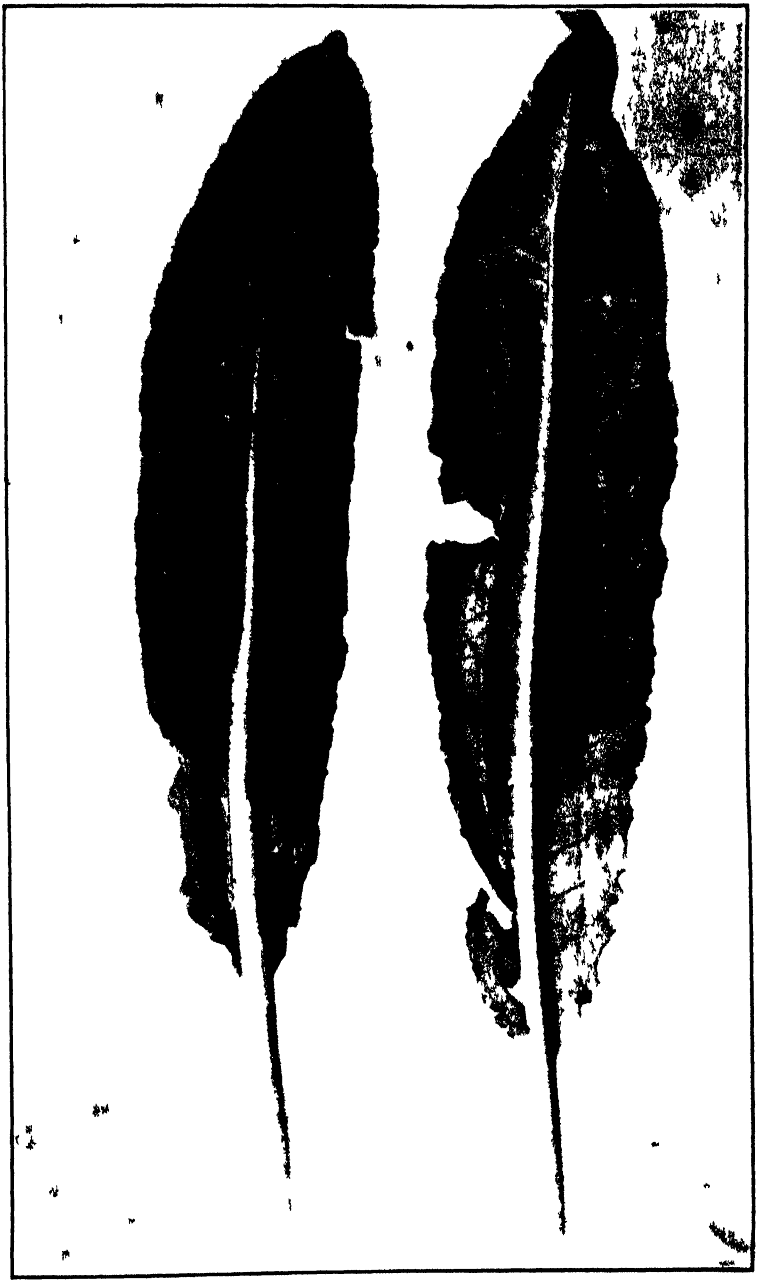
B. Sugar beets with mild form of curly-top produced by attenuated virus. On August 12, 1924, leafhoppers which had obtained the virus from a mildly affected beet (inoculated by a leafhopper from Bakersfield, Calif.) were caged on these ten plants, one insect to a plant. Photographed September 13, 1924. Note vein swellings (curly-top symptoms) on every plant, and compare size of plants with those in A.

PLATE XXXVII

A. Healthy sugar beet. Control for comparison with B and C.

B. A leafhopper with the virus from a severely affected curly-top beet was caged on this plant September 23, 1924. Photographed November 11, 1924.

C. Mildly affected curly-top sugar beet. A leafhopper with the attenuated virus was caged on this plant September 23, 1924. Photographed November 11, 1924.



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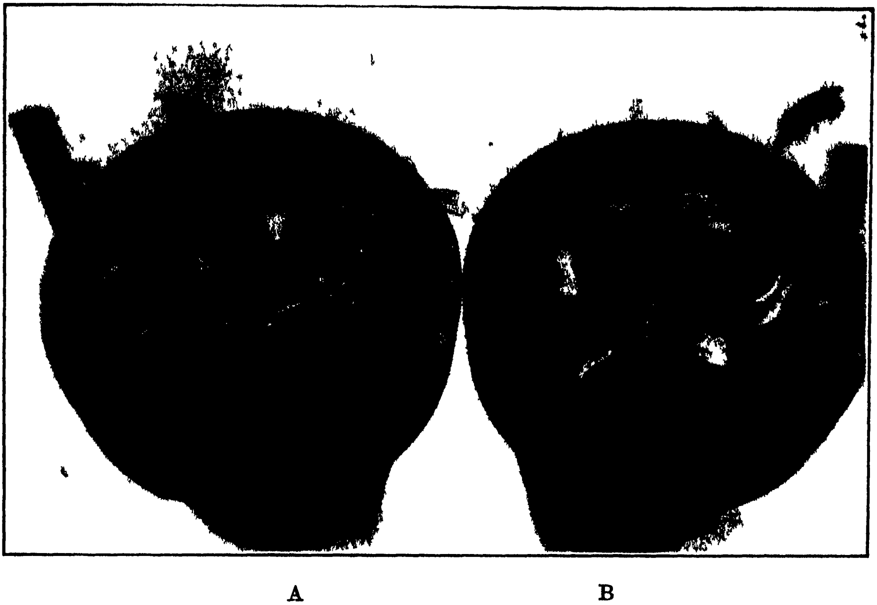
B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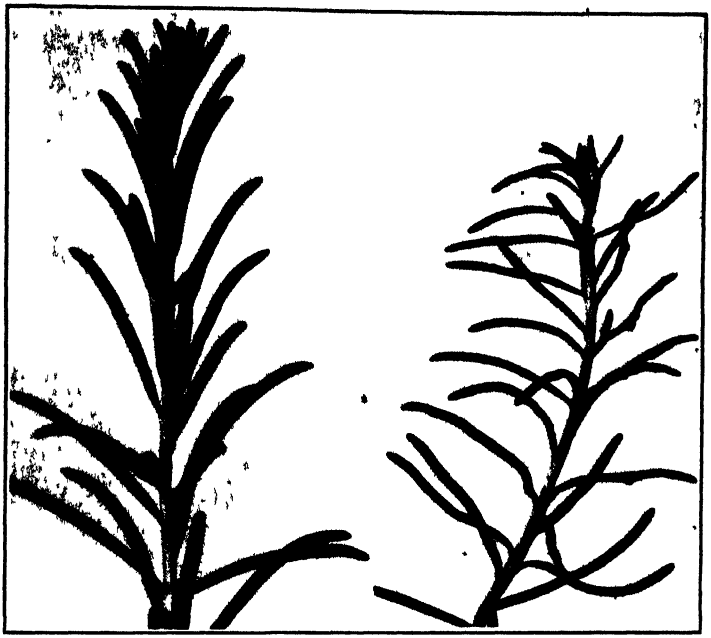

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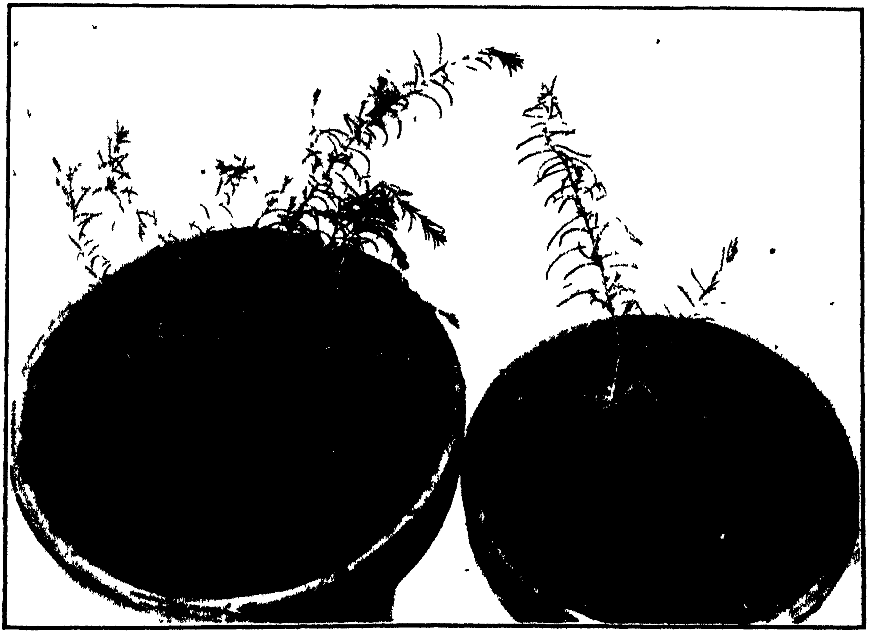

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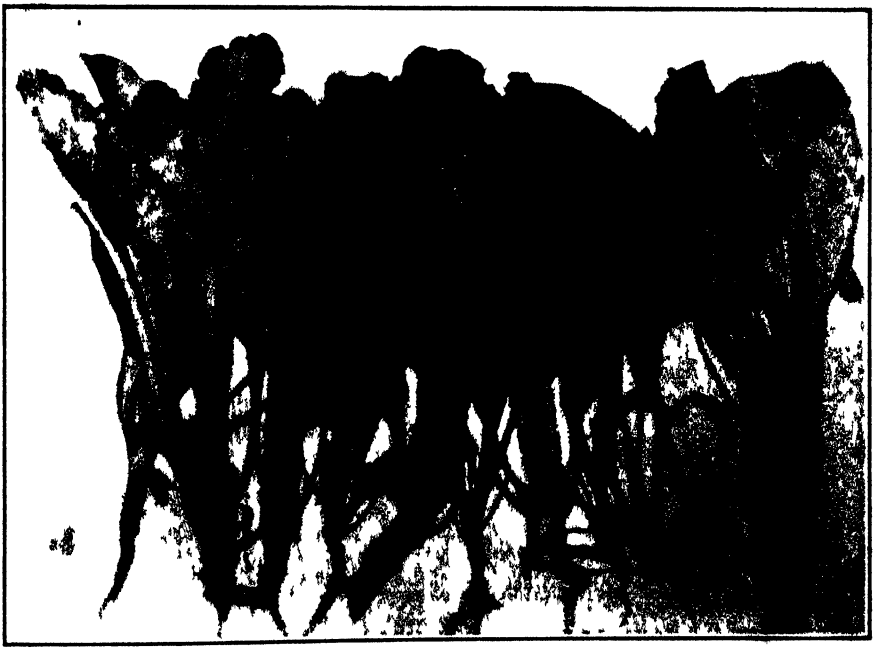

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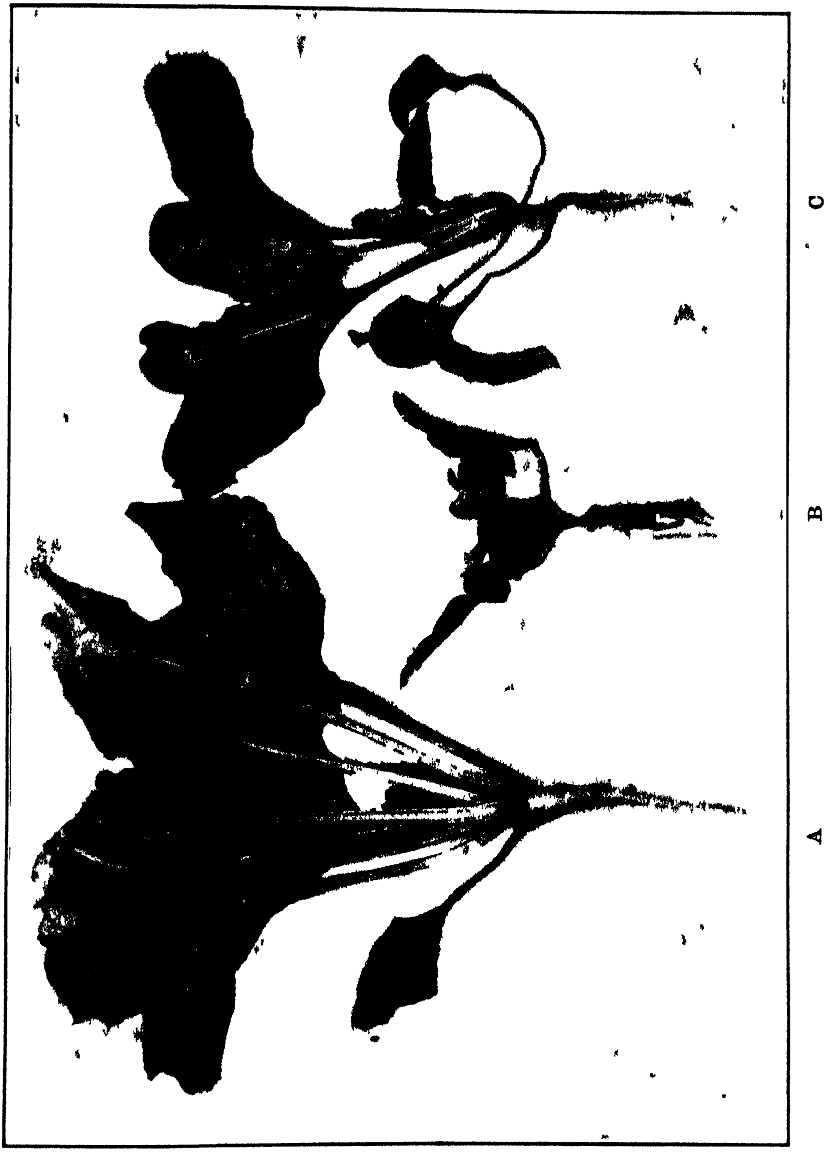
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HYPERPLASTIC CRUSHING OF THE TRACHEAL TUBES IN MOSAIC TOMATO STEMS[1]

MAX W. GARDNER[2]

WITH PLATES XXXVIII AND XXXIX

In a previous paper (3) it has been shown that the necrotic regions in the fruits of tomato plants affected with the severe streak or winter blight type of mosaic are frequently accompanied by a hyperplastic or proliferative response. In a partial review of the literature it was shown that internal as well as external necrosis was a very common symptom of the mosaic types of disease, and that hypertrophy and hyperplasia were sometimes associated with this necrosis.

The necrotic surface streaks on mosaic tomato stems, as well as internal necrotic lesions, have been described by several observers, including, in addition to those mentioned in the paper to which reference has been made, Vanterpool (7) in Canada. Inasmuch as a certain type of necrotic streaking of the stem in greenhouse tomatoes seems to be a reliable indication of the presence of mosaic, it is of interest to note that there are references to this trouble which antedate Bailey's (1) account. Galloway (2, p. 347), in 1889, cites a letter from a grower in which the necrotic symptoms of mosaic are described, and an earlier and more unmistakable record is contained in a similar letter quoted by Plowright (6) in 1887.

A microscopic study of these mosaic lesions has shown that the necrosis may occur in any of the tissues of the stem and petiole, and that frequently there is a vigorous hyperplastic response. Under certain conditions this hyperplastic or proliferative growth is so directed as to crush the tracheal tubes. Unstained razor sections of fresh material, and microtome sections of preserved material stained with Delafield's haematoxylin, have been studied.

The necrosis may occur in close proximity to the growing point in the very young regions of the stem. Hypertrophy of the adjacent cells may be present in this very young tissue, but extensive hyperplasia is found usually somewhat further back from the growing tip. This would indicate that the cell proliferation follows the necrosis and is a direct response to it.

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[1] Contribution from the Botanical Department of Purdue University Agricultural Experiment Station.

[2] The writer is indebted to Professor H. S. Jackson and Dr. E. J. Kraus for helpful suggestions and advice.

The necrotic pockets in the pith resemble those found by Kunkel in mosaic corn (4) and sugar cane (5). The affected cells collapse, and cavities of various shape and size are formed, each lined with the collapsed necrotic tissue. Hyperplasia of the adjacent pith cells occurs commonly, especially in very young pith (Pl. XXXIX, B) and when the necrosis is located near the outer regions of the pith. The outer regions of the pith contain the internal phloem strands, and the hyperplasia frequently originates near these strands in cases where there is no such response on the part of the pith cells lying on the inner side of the necrotic region. The hyperplasia in stems and petioles resembles that previously described in the fruit, and consists of zones, cushions, or whorls of thin-walled meristematic cells, devoid of intercellular spaces, growing in toward and crushing the necrotic cells.

Necrotic strips in the cortex, which constitute the externally visible brown streaks, may be internal or may extend to the exterior, and may result in surface grooves or crevices, owing to the collapse of the necrotic tissue. The necrosis may occur in the epidermis, the subepidermal cells, the collenchyma, or the cortical parenchyma. Necrotic zones in the inner portions of the cortex may be attended by hyperplasia in the shape of a cushion of thin-walled rectangular cells growing outward from the phloem region against the necrotic zone (Pl. XXXIX, D). This response was found most conspicuously developed in the base of the petiole. Necrotic surface streaks are frequently accompanied by hyperplasia of the underlying cortical cells, occasionally with the resultant formation of shallow surface blisters (Pl. XXXVIII, A) not unlike those previously described on the fruit.

The most vigorous hyperplastic response occurs, however, when the necrosis is in close proximity to the cambium, in which location plates of necrotic tissue, parallel to the cambial zone, seem to occur very frequently. These necrotic zones or planes are accompanied by a proliferative growth which apparently originates in the phloem region and pushes radially inward against the necrotic tissue and the xylem (Pl. XXXVIII, B). Under the hand lens, this growth is clearly visible as a glassy zone, measuring sometimes as much as 600 microns in radial thickness and bordered on the inner surface by a thin, brown, necrotic plane. The hyperplastic tissue is composed of short, thin-walled, closely packed cells quite unlike the xylem elements normally present in this region (Pl. XXXVIII, C; Pl. XXXIX, C). In longitudinal section, there occasionally has been observed an attempt at tracheal tube formation near the tangential edge of this hyperplastic tissue in the shape of an irregular row of short cells showing the characteristic wall thickenings.

These invasive hyperplastic growths are very similar to the internal intumescences found on the inner wall surfaces of the locules in mosaic fruits, except that they are considerably elongated in a direction parallel to the axis of the stem. In some cases, however, rather wide arcs of the cambial circumference are involved. As in the case of the fruit intumescences, it appears that the hyperplasia in the stem tissues is a response to the necrosis and that its occurrence depends upon whether or not the necrosis is located in or near reactive tissue.

These internal growths develop considerable inwardly-directed pressure, because they not only flatten the necrotic cells but also invade the xylem and crush the tracheal tubes which happen to lie in their path (Pl. XXXVIII, C, D; Pl. XXXIX, A). The lumina of such tubes are completely obliterated, so that the latter obviously can no longer function as channels for the passage of water. In cases where a considerable proportion of the circumference of the xylem cylinder is invaded by these tube-crushing hyperplastic growths, it is readily conceivable that the water supply to the distal portions of the stem might thus be cut off partially, and it seems possible that this may account for certain effects of the disease, such as the wilting of young leaves.

SUMMARY

The severe streak or winter blight type of tomato mosaic is characterized by necrotic strips and pockets in all of the tissues of the stems and petioles.

This necrosis is frequently accompanied by hyperplasia or proliferation of the adjacent cells in the shape of zones or cushions of muriform tissue pushing in against the necrotic tissue.

When necrotic planes occur near and parallel to the cambial region, the inwardly-directed hyperplastic response is often so vigorous as to invade the xylem and flatten and crush the tracheal tubes.

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EXPLANATION OF PLATES

PLATE XXXVIII

A. Surface blister on mosaic tomato stem caused by hyperplasia of subepidermal cells underneath an area of necrotic epidermal cells. Longitudinal section, enlarged × 79.

B. Cross section of mosaic tomato stem showing necrotic planes in the xylem region pressed inward by the hyperplasia originating apparently in the phloem region. Enlarged × 30.

C. Central part of B enlarged × 74 to show the muriform, thin-walled tissue of the hyperplastic growth, and the flattening and crushing of the tracheal tubes upon which the abnormal growth impinges.

D. Cross section showing abnormal hyperplastic growth pushing inward against a flattened plane of necrotic tissue and crushing the tracheal tubes. Enlarged × 86.

PLATE XXXIX

A. Cross section showing early stage of the crushing of the tracheal tubes by a hyperplastic growth. Groups of normal tracheal tubes are visible at the extreme right and left sides. Enlarged × 92.

B. Cross section of petiole showing necrotic zone in the pith accompanied by hyperplasia of the surrounding pith cells. Enlarged × 40.

C. Longitudinal section of the hyperplastic tissue pushing inward from the left against a necrotic plane and crushed tracheal tubes at the right. The cells are more or less rectangular and are not greatly elongated. Enlarged × 114.

D. Section through cortex near base of petiole showing a necrotic pocket in the cortex and a hyperplastic cushion pushing outward and crushing the necrotic tissue. Enlarged × 53.

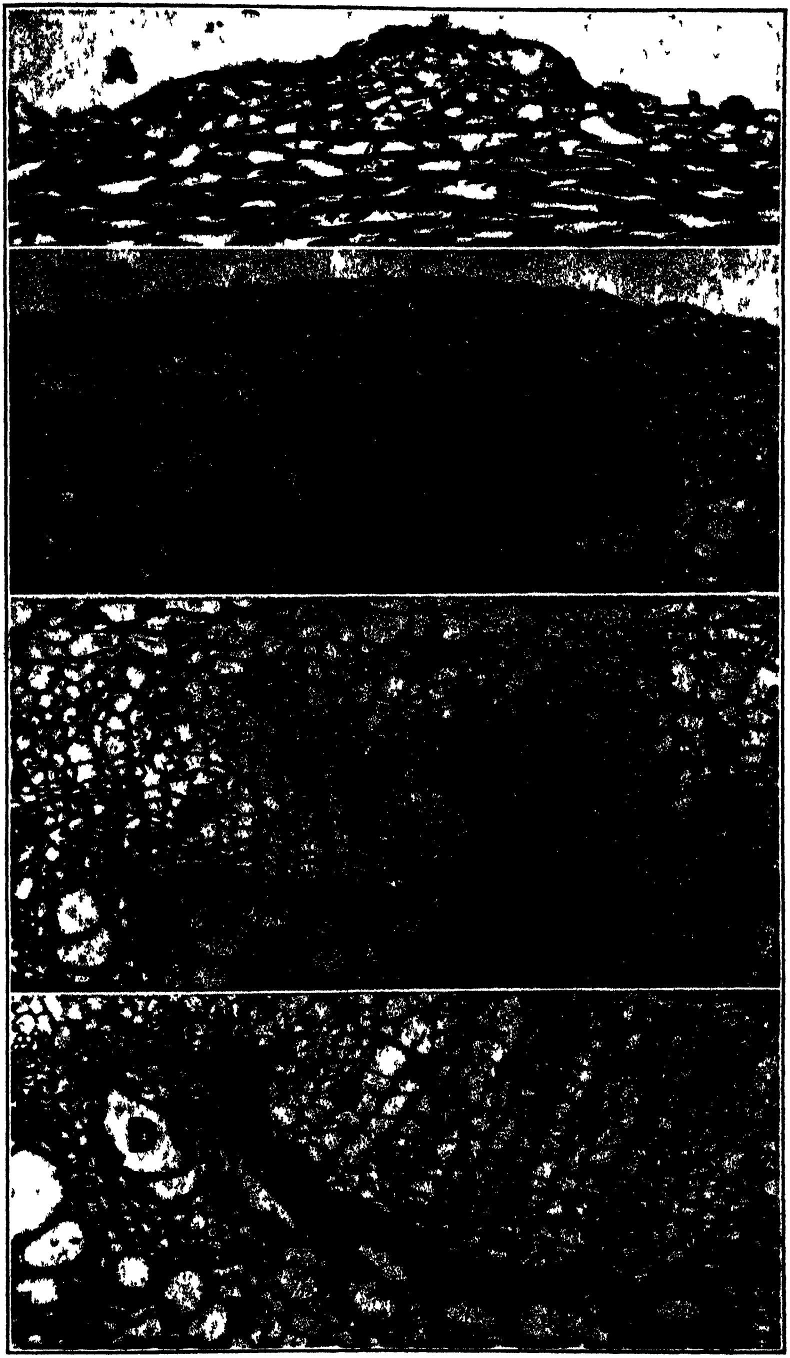
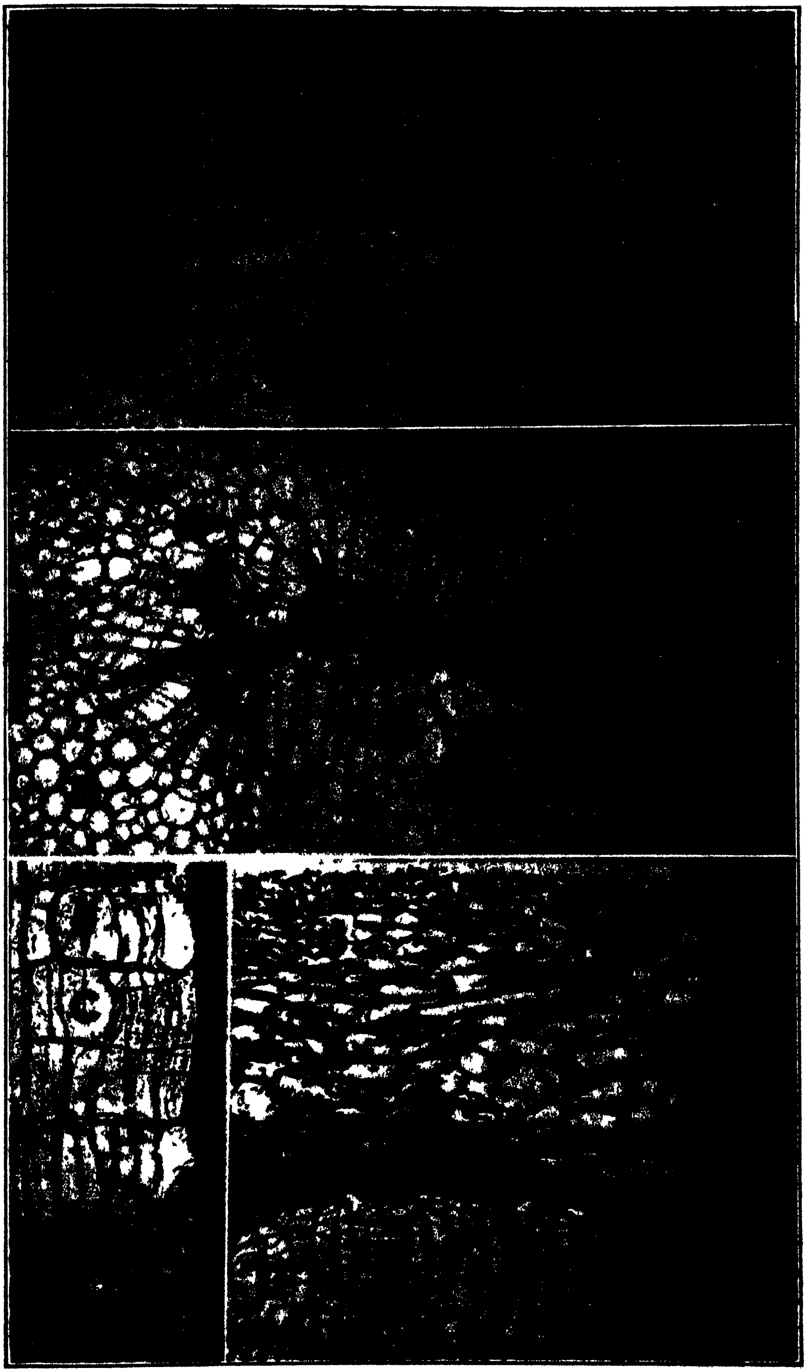


PLATE XXXIX



THE MOSAIC DISEASE IN THE GARDEN PEA AND OTHER LEGUMES

S. P. DOOLITTLE AND F. R. JONES

WITH PLATE XL

INTRODUCTION

Field observations of the past two years have shown that the mosaic disease of the garden pea (*Pisum sativum*) is widely distributed in Wisconsin. This disease was reported by Dickson (2) in 1922 as occurring in Quebec, and was also noted by Martin and Haenseler (5) in New Jersey in 1924. Mosaic plants were first observed at Madison, Wis., in 1923, and the disease was observed in many commercial fields in the state during 1924. Although it occurred in experimental plats at Madison in the latter part of June, the disease was not generally conspicuous in the field until about July 15. The pea disease survey (4) records it in 63 fields in various parts of Wisconsin. Many of these fields showed only a trace of the disease, but a considerable number contained from 5 to 15 per cent of mosaic plants. In general, although certain varieties seemed to suffer slightly from the disease, mosaic cannot be considered at present a serious menace to peas grown for the canning industry. A considerable amount of mosaic also occurred in 1924 on all varieties in the extensive pea trial grounds of Dr. W. B. Brotherton at MacMillan, Mich. Dr. Brotherton reports that late season varieties were severely damaged.

Among other legumes which show mosaic is the sweet pea (*Lathyrus odoratus*). Almost all of the sweet peas observed in the vicinity of Madison in 1923 and 1924 were infected with mosaic and severely damaged. The cultivation of this plant seems to have been generally abandoned in gardens at Madison because of this disease. Since the most common legume upon which mosaic occurs in gardens and near pea fields appears to be the red clover (*Trifolium pratense*)—a perennial upon which the disease overwinters—it seemed likely that the mosaic diseases upon these hosts might be intertransmissible. This paper is a record of studies of mosaic in these three species.

SYMPTOMS OF MOSAIC IN THE GARDEN PEA AND SWEET PEA

In the early study of mosaic in the garden pea, difficulty was sometimes experienced in distinguishing between certain distorted conditions of foliage occurring both in the greenhouse and field and true mosaic. Plants injured by frost sometimes produce pale or crinkled foliage, suggesting a disease of the mosaic type. The mosaic studied here on the garden pea is characterized by a distinct mottling of the leaves, but there is little of the curling, wrinkling and general deformity of the leaf which occurs in the sweet pea and in mosaic plants of some other families. Mosaic pea leaves are usually a lighter green than those of normal plants and, in most cases, the mottled appearance is due to the presence of numerous small, dark green areas which occur between the larger veins (Pl. XL, fig. A). These dark areas are irregular in outline and usually seem to follow the small veinlets, but there appear to be none of the large green areas which occur in the case of such diseases as tobacco mosaic. Where plants have been mosaic for some time, the symptoms are often of a different type. In such cases, the dark green portions of the leaves may be replaced by yellowish-green areas which are of similar size and outline but which are lighter in color than the rest of the leaf.

In general, the leaves of mosaic peas are somewhat smaller than those of normal plants, and occasionally there is some slight curling of the edges of the younger leaves. Both symptoms appear most pronounced in the later varieties of more luxuriant growth. Such plants are occasionally considerably dwarfed by the disease, particularly if infected when small. In such cases, the pods appear to be smaller and fewer than those of healthy plants of the same varieties. The incubation period of the disease seems to vary from 6 to 14 days.

The mosaic disease of the sweet pea differs from the disease on the garden pea in that the plants are much more severely affected. The disease appears first in the younger leaves, and the symptoms at this stage are much like those of the garden pea. Occasionally, however, the contrast is greater between the dark areas and the remainder of the leaflet, which becomes a light greenish-yellow (Pl. XL, fig. C). As the disease progresses, the younger leaves develop a pronounced upward curling of the edges, which gives them a rolled appearance. Such leaves commonly develop small, elongated areas which are light yellow and appear to be thinner than the remainder of the leaf. These areas may be either raised or depressed, usually the latter, and appear much like the results of insect injury. Mosaic sweet peas are often much stunted in growth, not only of tops but of roots; and the plants sometimes appear to die of rootrot. The blossoms are streaked and paler than those of normal plants. A comparison made in 1924 showed that 82 mosaic plants produced only 85 blossoms in a period of 3 weeks from the time the first flowers appeared, while 38 healthy plants of the same varieties produced 187 blossoms in the same period.

INOCULATION EXPERIMENTS WITH MOSAIC OF THE GARDEN PEA

The inoculation experiments with the garden pea were conducted chiefly in the field, using the Alaska, Telephone, and Eclipse varieties. All of the plants inoculated were grown under cages covered with cheesecloth in order to prevent chance infection by insects. The inoculations were made by artificial methods and by means of aphids. In the artificial inoculations, the leaves and stems of mosaic pea plants were crushed in a sterilized mortar and used as inoculum. Inoculations were made from peas found infected in the field and also from plants experimentally infected from this source. The inoculations were made by pricking a few drops of the mosaic juices into 4 to 6 of the younger leaflets and by inserting a small fragment of crushed leaf tissue in a slight incision near the base and at the tip of the stem. The control plants were treated in the same manner, using the juices of healthy pea plants. All of the controls were in the same cages with the plants inoculated. In the aphis inoculations, pea aphids [*Illinoia* (*Macrosiphum*) *pisi* (Kalt.) Baker] were transferred from mosaic pea plants to the leaves of healthy peas under cages. Approximately 10 aphids were placed on each plant, and an equal number from healthy peas were transferred to each of the control plants, which were under a separate cage. These experiments are summarized in table 1.

TABLE 1.—*Results of inoculations to garden peas from peas affected with mosaic*

Date	Method of inoculation	No. plants inoculated	No. plants mosaic	Date observed
6/17/24	Artificial	13	5	6/30/24
do	Control	10	0	7/10/24
6/26/24	Artificial	27	8	7/ 8/24
do	Control	16	0	7/15/24
7/ 3/24	Aphids	30	23	7/18/24
do	Control	24	0	7/25/24
8/12/24	Artificial	20	10	8/30/24
do	Control	17	0	9/ 5/24
8/19/24	Aphids	16	16	8/29/24
do	Control	21	0	9/ 3/24

All of the varieties of peas used were susceptible to the disease, but the Telephone seemed more easily infected than either the Alaska or the Eclipse. In the inoculations with the Telephone pea, 70 per cent of the plants were infected, while with the Alaska and the Eclipse only 40 per cent of the plants became diseased. The transmission by aphids was also indicated in certain field plants which showed only a trace of mosaic until about 15 days after they became heavily infested by aphids, when a high percentage of the plants showed mosaic.

INOCULATION EXPERIMENTS WITH SWEET PEA MOSAIC

Taubenhaus (6), in 1914, reported the occurrence of sweet pea mosaic in Delaware and described a series of experiments which indicated that it was transmissible either by artificial inoculation or by aphids. A series of experiments by the writers has confirmed his results. These experiments consisted of artificial inoculations made in the same manner as described for the garden pea and also of experiments on the transmission of the disease by the pea aphis. The plants used in these experiments were grown in cages large enough to permit the worker to enter. Four varieties of sweet peas were used in the experiments: Glitters, Mrs. Cuthbertson, Flamingo, and Royal Scott, all of which proved susceptible to the disease. These experiments, as shown in table 2, have indicated that sweet-pea mosaic is transmissible either by aphids or by artificial inoculations.

TABLE 2.—*Results of inoculations to sweet peas from sweet peas affected with mosaic*

Date	Method of inoculation	No. plants inoculated	No. plants mosaic	Date observed
6/16/24	Aphids	63	50	6/28/24
do	Control	50	0	7/10/24
6/17/24	Artificial	20	12	6/28/24
do	Control	22	0	do
8/13/24	Artificial	10	6	8/25/24
do	Control	10	0	8/30/24

CROSS-INOCULATIONS FROM MOSAIC SWEET PEAS TO THE GARDEN PEA

Cross-inoculations, both by means of the pea aphis and by the use of the expressed juices of mosaic plants, have shown that sweet pea mosaic is transmissible to the garden pea. The Telephone and the Eclipse varieties were used under cages in the field. The results are given in table 3.

CROSS-INOCULATIONS FROM MOSAIC GARDEN PEAS TO THE SWEET PEA

Dickson (2) reported successful cross-inoculations from the garden pea to the sweet pea in 4 out of 28 plants, but did not draw definite conclusions from the experiment. The writers have found that the sweet pea is readily infected with the mosaic occurring on the garden pea. Thirty plants were inoculated by means of aphids taken from mosaic garden peas, 28 of which developed mosaic within 15 days. Aphids from healthy peas were placed on 20 other plants as controls, all of which remained healthy. Artificial inoculations of 38 plants resulted in infection in 20 cases. The results of the above cross-inoculations have shown that the diseases of the garden pea and sweet pea are intertransmissible, and, since the symptoms produced on these hosts when inoculated from either the garden pea or sweet pea appeared to be the same, no distinction has so far been found between the diseases on these two plants.

TABLE 3.—*Results of cross-inoculations to garden peas from mosaic sweet-pea plants*

Date	Method of inoculation	No. plants inoculated	No. plants mosaic	Date observed
2/27/24	Aphids	23	11	3/14/24
do	Control	13	0	do
do	Artificial	19	0	3/19/24
do	Control	13	0	do
6/10/24	Aphids	32	22	6/21/24
do	Control	31	0	6/30/24
6/26/24	Aphids	31	16	7/ 8/24
do	Control	29	0	7/10/24
7/ 3/24	Artificial	16	8	7/15/24
do	Control	15	0	do
8/19/24	Aphids	37	35	8/30/24
do	Control	39	0	do
do	Artificial	18	10	do
do	Control	15	0	do

MOSAIC RED CLOVER AS A SOURCE OF INFECTION TO THE GARDEN PEA AND SWEET PEA

The frequent occurrence of mosaic on red clover has been mentioned previously. Davis (1) states that this perennial plant harbors the pea aphis during the winter and that the aphids migrate from the clover to pea fields in the early summer. If these migrating aphids can transmit mosaic from red clover to peas, the presence of mosaic in the pea fields thus can be readily accounted for. Field observations have indicated that the mosaic in red clover is transmissible to peas. In 1924, a number of rows of peas were planted parallel to a plat of red clover in which a large number of the plants were affected with mosaic. Observations made early in July showed that 96 per cent of the peas were affected with mosaic in the row adjoining the clover. In the next row the infection on the peas was somewhat less, and the reduction in the amount of mosaic continued in each succeeding row, the fourth showing only 50 per cent of mosaic. It seemed probable that the row adjoining the clover was more severely affected with mosaic as a result of the greater ease and frequency of the aphis migration from the clover, but data of this sort are, of course, merely suggestive. Inoculations were made, therefore, to determine the susceptibility of the garden pea and the sweet pea to the mosaic on red clover.

CROSS-INOCULATIONS FROM MOSAIC RED CLOVER TO PEAS

Most of the inoculations from red clover to peas were made by the artificial method, using the same technique described in the case of other inoculations. The plants were grown in the field, and the controls were in the same cages with the inoculated plants. Two series of inoculations also were made with aphids. In this case the aphids from a cage of healthy peas were colonized on mosaic red clover plants in the greenhouse. After 24 hours the insects which remained on the clover were transferred to peas under a cage in the field. Aphids from healthy clover plants were placed on other pea plants as controls. The results of these experiments, as shown in table 4, indicate that the mosaic of red clover is transmissible to the garden pea either by means of insects or by artificial inoculation. It seems probable, therefore, that red clover acts as a source of mosaic infection to peas in the field.

TABLE 4.—*Results of inoculations to garden peas from mosaic red clover plants*

Date	Method of inoculation	No. plants inoculated	No. plants mosaic	Date observed
6/24/24	Artificial	18	6	7/ 8/24
do	Control	17	0	7/15/24
7/ 3/24	Artificial	17	8	7/14/24
do	Control	14	0	7/23/24
8/12/24	Aphids	10	7	8/22/24
do	Control	9	0	8/30/24
do	Artificial	24	12	8/23/24
do	Control	20	0	8/30/24
8/17/24	Aphids	8	6	8/28/24
do	Control	8	0	8/30/24
8/21/24	Artificial	27	11	9/ 6/24
do	Control	29	0	9/10/24
8/27/24	Artificial	23	8	do
do	Control	18	0	do

CROSS-INOCULATIONS FROM MOSAIC PEAS TO RED CLOVER

Some evidence has been secured in relation to the transmission of pea mosaic to the clover by aphids. Healthy red clover plants were grown in a cage with peas which were afterwards inoculated with pea mosaic.

Aphids were introduced into the cage after mosaic had developed on the peas, and a certain number of the insects migrated from the peas to the clover. At the end of 4 weeks, 6 out of 14 clover plants showed mosaic. Twelve healthy clover plants in another cage with healthy peas showed no signs of mosaic during the season, although aphids were present. Similar experiments are in progress.

CROSS-INOCULATIONS FROM MOSAIC RED CLOVER TO SWEET PEAS

As in the case of the garden pea, it has been found that the sweet pea is susceptible to the mosaic occurring on red clover. The inoculations in this case were made only by the artificial method and included 62 plants, 14 of which developed mosaic, while all of the 60 controls remained healthy. No inoculations have been made from the sweet pea to the red clover by the artificial method, but there is evidence that the pea aphis can transmit sweet-pea mosaic to this host. Healthy red-clover plants were grown in a cage of sweet peas which were inoculated with sweet-pea mosaic by means of aphids. After the aphids had been present for a number of weeks, it was found that 3 out of 10 of the clover plants had developed mosaic. Healthy clover plants in another cage of healthy sweet peas which were also infested with aphids showed no evidence of mosaic throughout the season.

TRIALS WITH SEED OF MOSAIC PEA PLANTS

Dickson (2) reported seed transmission of pea mosaic in certain varieties grown in the greenhouse, and, in a later abstract (3), mentioned further evidence of seed transmission in other varieties. In order to secure further evidence on this point, trials were made with seed collected by Dr. Brotherton at MacMillan, Mich., from 27 plants of the following varieties: Duchess of York, Prince Edward, Telephone, Carter's Daisy, Sharpe's Standard, and Eclipse. One hundred and sixty-two plants were grown in the greenhouse. The seed was planted on February 28, but no mosaic was noted on any of the plants until March 27, when they had reached a height of about 8 inches. On this date 7 plants showed signs of mosaic, but, since the disease had not appeared on the older leaves, it seems likely that infection came from other legumes in the houses, particularly since a few aphids had appeared in spite of efforts to control them by fumigation. During the spring of 1924, the remainder of this seed was grown under cheesecloth cages in the field. The seed was planted on June 15 and was covered with cages before the plants emerged. Four hundred and ninety-three plants were grown practically to maturity, none of which showed any evidence of mosaic.

In addition to this, another trial was made later in the season with seed collected from mosaic plants grown during 1924. The seed was collected from plants of the Alaska and the Ironclad field pea which developed practically all of this seed after their infection with mosaic. The seed was planted on August 16 under frames which were covered with cheesecloth. Due to the unusually cool moist summer, the plants made a vigorous and rapid growth and appeared to be little affected by the slight shading of the cloth. On September 11 the covering was removed and the plants examined for evidences of mosaic. At this time they had reached a height of 12 to 14 inches, but none of the 1,038 plants of Alaska and 388 plants of Ironclad showed any signs of mosaic. In order to make sure that the symptoms of mosaic had not been masked by the shading of the cheesecloth, the covers were not replaced after examination of the plants and they were left in the open until September 28 when they were again examined. On this date there was still no sign of mosaic infection. The experiments to date, therefore, have comprised 1,919 plants grown from seed from mosaic plants under insect-proof cages without the production of a single mosaic plant. These experiments are being continued on a larger scale in 1925.

CROSS-INOCULATION EXPERIMENTS WITH MOSAIC DISEASES OF OTHER LEGUMES

Cross-inoculations have been made from mosaic sweet clover (*Melilotus alba*) and bean (*Phaseolus vulgaris*) to garden peas and sweet peas without success. Further trials are in progress. The unsuccessful inoculations are summarized in table 5.

TABLE 5.—*Summary of unsuccessful cross inoculations from mosaic sweet clover and bean to the garden pea and sweet pea*

Date	Plant used as inoculum	Plant inoculated	No plants inoculated	No plants mosaic	Date observed
2/27/24	Sweet clover	Garden pea	26	0	3/14/24
7/ 3/24	do	do	13	0	7/20/24
7/16/24	do	Sweet pea	22	0	8/ 7/24
8/12/24	do	Garden pea	16	0	9/17/24
8/21/24	do	do	19	0	do
do	do	Sweet pea	16	0	do
8/14/24	Bean	do	19	0	do
do	do	Garden pea	21	0	do
8/20/24	do	do	17	0	do

SUMMARY

1. The mosaic of the garden pea is widely distributed in Wisconsin, but it appears to cause little injury except in late season varieties. Sweet

peas, however, are severely damaged by mosaic. The symptoms of the two diseases are similar, but there is a greater mottling, dwarfing, and distortion of the leaves in the sweet pea.

2. The mosaic diseases of both the garden pea and sweet pea are transmissible by the pea aphis and by artificial inoculations. The mosaics occurring on these hosts are also intertransmissible by either method of inoculation.

3. Red-clover mosaic has been transmitted to the garden pea and sweet pea by artificial inoculations and to the garden pea by means of aphids. Inoculations by means of aphids also have indicated that red clover is susceptible to the mosaic occurring on these two hosts.

4. Since the red clover is a perennial on which the pea aphis is said to overwinter, it is probable that the aphids migrating from clover to peas in the spring act as carriers of the disease and thus introduce mosaic into the fields.

5. Over nineteen hundred plants have been grown from seed of mosaic garden and field peas, but there has been no evidence of seed transmission of the disease.

6. Cross-inoculations to garden peas and sweet peas from mosaic bean and sweet-clover plants have yielded only negative results.

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DISEASE INVESTIGATIONS,
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UNITED STATES DEPARTMENT OF AGRICULTURE.

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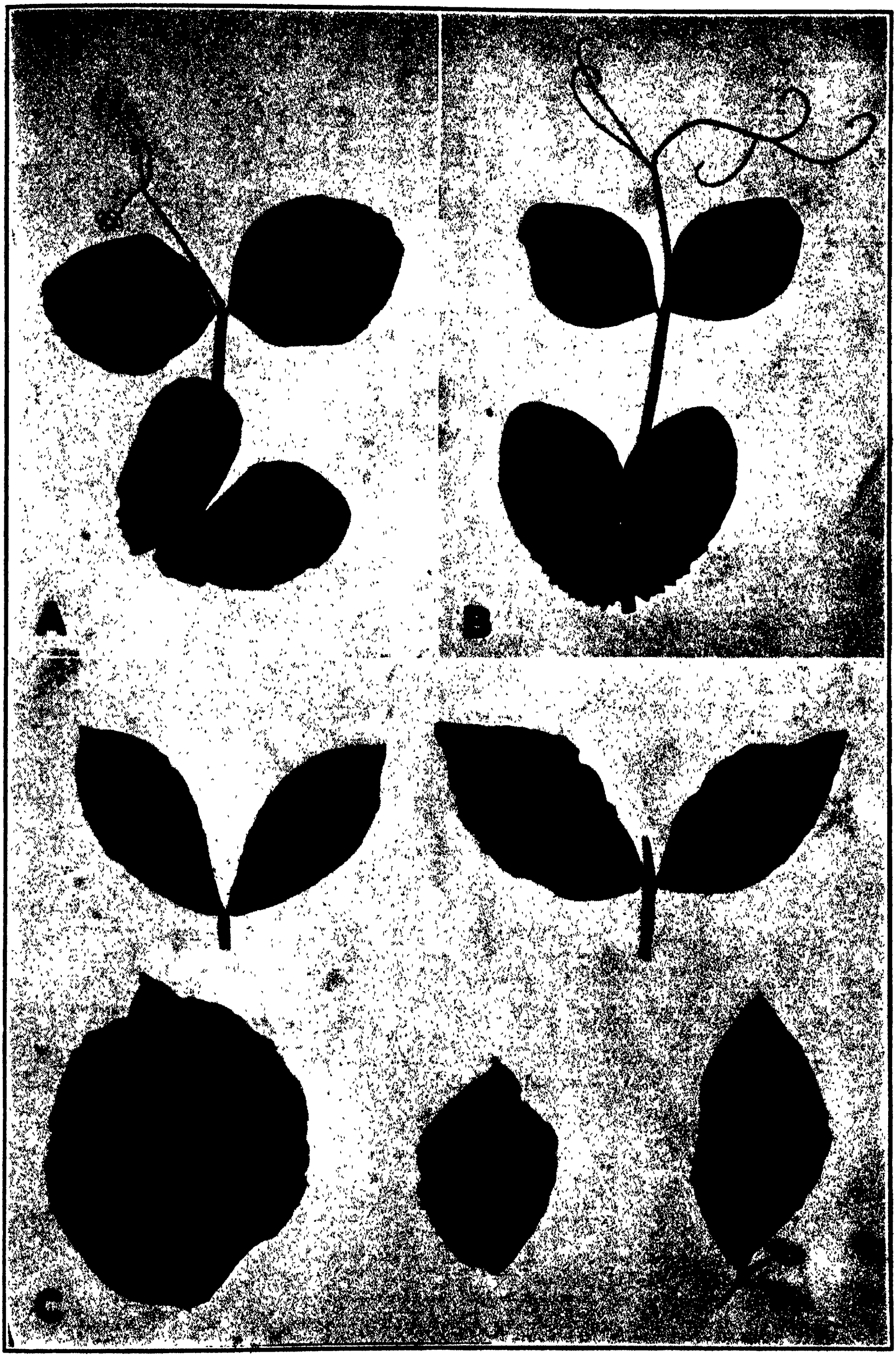
EXPLANATION OF PLATE XL

A. Mosaic pea leaf showing typical symptoms.

B. Healthy pea leaf.

C. Mosaic and healthy sweet pea leaflets showing various types of symptoms. Healthy leaflet in upper right corner.


A
B
C

ANTAGONISM OF THE WALNUTS (JUGLANS NIGRA L. AND J. CINEREA L.) IN CERTAIN PLANT ASSOCIATIONS

A. B. MASSEY

WITH FIVE FIGURES IN THE TEXT

The writer's attention has frequently been directed to the poor development and dying of tomatoes and other plants in the immediate vicinity of walnut trees. This is especially true of the black walnut, *Juglans nigra,* which is found in more frequent associations with crop plants than the butternut, *Juglans cinerea.* Review of the literature on the subject has not revealed any report of a definite study, but there are several notes which bear directly on the matter. Jones and Morse (2), in a study of the shrubby cinquefoil, *Potentilla fructicosa* L., a pasture pest in Vermont, record observations made by A. H. Gilbert on the effect of butternut on the cinquefoil. It was observed that around butternut trees there was a circular area, greater than the spread of the limbs, in which the cinquefoil was dead. At the borders of the area, dying plants were found. The death of the cinquefoil always indicated that the trouble had its origin in the roots. Examination of the root systems of dead and dying plants revealed in every case a close association between the roots of the weed and those of the butternut. In situations where rock outcrop interfered with the spread of the butternut roots, the cinquefoil grew normally.

> "Moreover, with such butternuts, the 'dead line' for the weeds is pushed outward year by year as the tree enlarges, so that the trees may be surrounded by a circle of dead and dying cinquefoil plants bordering the clean grassy plot under the tree. This antagonism is, we believe, attributable rather to the root relations of the two plants, than to those of shade. Thus young butternuts from 2 to 8 feet high were observed to be surrounded by a circle which might be twice the diameter of the top of the tree, within which the weeds were dead, with dying plants bordering its margins. Such butternuts do not cause much shade. Moreover, young birch, beach, maple, cherry, apple and pine trees in the same field showed no such striking relation to the death of the cinquefoil, healthy plants of the weed frequently growing close under their branches." (2, p. 189.)

In a short note, Mel. T. Cook (1) mentions having observed several cases of injury to tomatoes and potatoes in the near vicinity of black walnut trees. He writes:

> "Attention has been called from time to time to a number of cases of wilting of potato and tomato plants which was undoubtedly due to walnut (*Juglans nigra*) trees growing in the immediate vicinity. The plants show a pronounced wilting but

> do not lose their color or die, as in the case of plants that have been attacked by wilt-producing fungi or bacteria, or struck by lightning. The range of the wilting coincides very closely with the spread of the root system. The plants may wilt uniformly within a large circle or there may be areas of wilted and areas of erect plants, which coincides with the distribution of the root system. In some cases the wilting was in a circular area around the trees in the field, in other cases in a semi-circular area next to trees along the margins of the fields. In all cases observed, the plants beyond the spread of the roots of the trees were normal. A number of cases have been investigated by the writer and there is no doubt as to the cause of the wilting. So far as the writer has observed, other crops are not affected by the walnut trees and other trees do not cause a wilting of crops or wilt vegetation.''

Dr. A. W. Drinkard, Jr., Director of the Virginia Agricultural Experiment Station, has described to the writer orally the severe injury to experimental plats of tomatoes which, by chance, were planted near some black walnut trees on the edge of a field. When about half grown, the tomato plants wilted and died over a semi-circular area extending 30 to 40 feet from each walnut tree as a center. On one side of the plats was a small stream which kept the soil not only well supplied with water but was inclined to keep it too wet. It is evident from this observation that the effect of the walnut is not a case of water relation as might be suggested.

Fromme, in an unpublished note, describes disastrous results to tomatoes in Virginia:

> ''On several occasions in Virginia a wilting of tomato plants growing in proximity to black walnut (*Juglans nigra*) has been noted. The first observations were made near Amsterdam in 1916. Areas of wilted plants were seen in two separate fields of tomatoes, and each area centered on a walnut tree growing in the fence row. The rapid wilting of the plants suggested bacterial wilt (*B. solanacearum*), but examination failed to reveal the presence of this organism. On inquiry it was learned that the occurrence of this type of wilt in proximity to walnut trees was a matter of common observation among farmers in the locality.
>
> ''An opportunity for additional observations came during the season of 1917 in connection with a spraying experiment at Blacksburg. A number of small plots were provided in this test, each consisting of 16 plants set in the form of a square. The spacing between plants was 5 feet, with 7 foot alleyways between plats. The arrangement is shown in figure 1, together with the location of two black walnut trees, on the border of the field, which were overlooked at the beginning of the work. The varieties were Stone and Greater Baltimore. The date of transplanting was May 22; and the first wilting was noted approximately two months later, on July 26. The number of wilted plants increased rapidly as the season advanced, and by harvest all plants within the zone of influence of the trees showed marked injury. The majority of the plants were dead at this time, and fruit production throughout the areas affected was practically nil. The location of affected plants as determined at harvest time is shown in the diagram by means of the blackened squares. It will be noted that all plants within a radius of 50 feet of one tree and of 40 feet of the other were affected. There were 42 affected plants in the first area and 20 in the second.

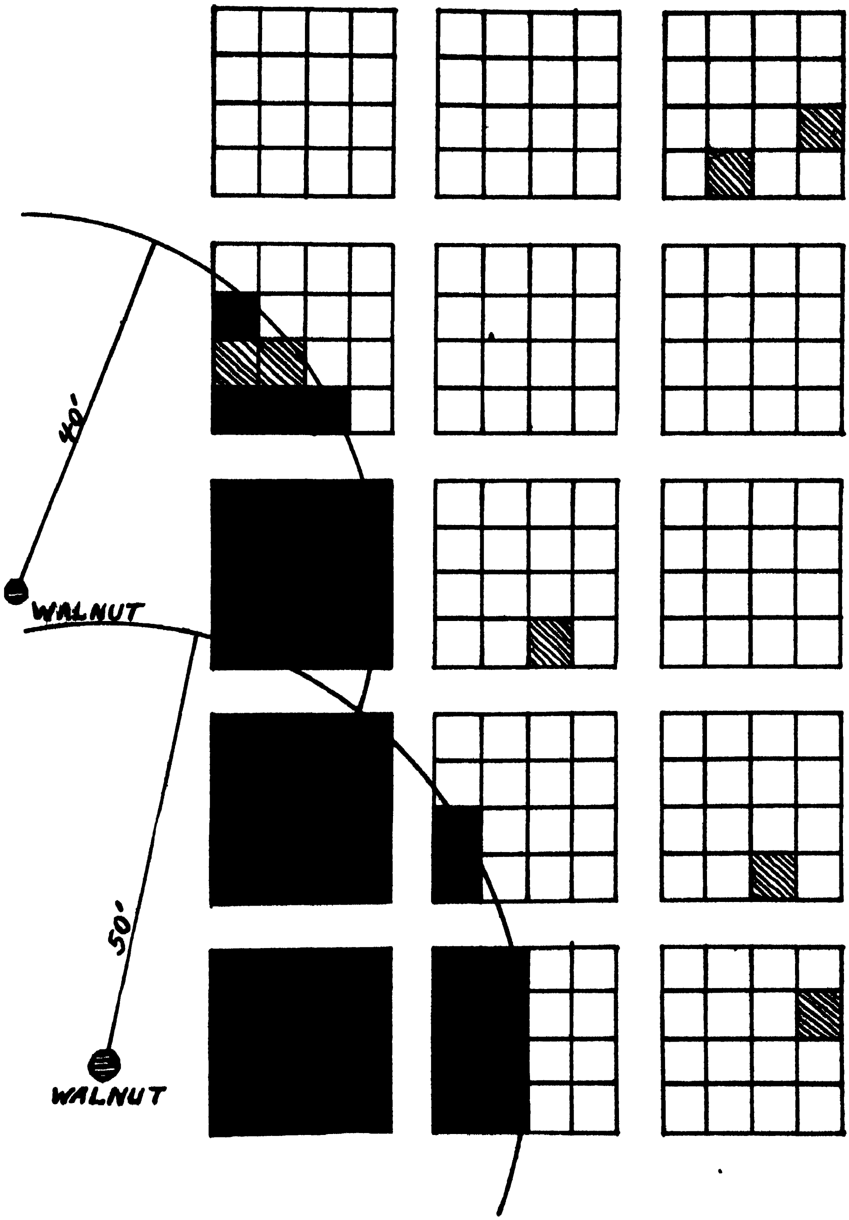


FIG. 1. Map showing location of wilted tomato plants with reference to black walnut trees. Black squares show wilted plants, white squares healthy plants, shaded squares missing plants. (Fromme.)

> "The writer has made no study of the cause of this wilting, but the evidence suggests the effect of a toxic excretion from the walnut roots rather than a depletion of soil moisture. As Cook has noted, plants other than tomato and potato are not affected, and trees other than walnut apparently do not cause a similar effect."

The majority of the observations the writer is able to find relate to the effect of the walnut on tomatoes and potatoes. These plants seem to be especially susceptible. In an unpublished report for 1922 of the field laboratory for fruit disease research, located at Winchester, Va., F. J. Schneiderhan reports a case of injury to four apple trees equi-distant on as many sides of a black walnut tree. His note is as follows:

> "A noteworthy case of incompatibility between trees of different species was observed in the experimental plats of the Stonewall Orchard. The first apple tree in Plat 1 of our scab experimental work was found to have dead branches on its west side, No cankers or other causes were found on the tree itself. A large black walnut tree stands 50 feet west of this apple tree. It was thought possible that a toxic influence of the *Juglans* species might have some connection with the killing of the branches mentioned. Upon investigation, it was discovered that other apple trees planted north, east and south of this same walnut tree were affected to a greater extent than the tree in our experimental plat. The tree located south of the walnut tree was a dwarf, the one on the north had not only lost all of its branches on the side facing the walnut tree but was also severely dwarfed, while the apple tree on the east was completely killed. From this we concluded that black walnut trees planted within 50 feet of apple trees exert a toxic influence which results in severe dwarfing and ultimate death of the latter."

Unverified reports of similar cases of injury to apple trees in other orchards in Virginia have been brought to the writer's attention.

Schreiner and Reed (3) state, "It is known that certain crops do not thrive when planted on newly cleared land which was originally covered with walnut or butternut trees." It is evident from these few authentic observations which the writer has been able to locate that there is a decided antagonism of species of *Juglans* to plants of different types. However, no one seems to have made a close study of the matter to determine the cause.

Antagonism in other plant associations has been noted by Schreiner and Reed (3), and by Schreiner and Skinner (5). These investigations will not be discussed here.

With the evidence in mind of an antagonism between the roots of *J. cinerea*, *J. nigra*, and certain weed and crop plants within their reach, the writer in 1923 decided to investigate the matter in the hope of obtaining some data as to the cause of the antagonism. Several possible explanations came to mind when planning the approach to the problem.

1. Is there an exhaustion of the soil water by the walnut trees, thereby causing wilting and death of the plants affected?
2. Is there a toxic substance developed in the soil from the decay of walnut leaves or nut shells around the tree?
3. Do the roots secrete a toxic substance which is detrimental to some plants?

The first and second questions were readily disposed of after careful consideration of the authentic cases described above. The condition of the soil in the tomato plats of Drinkard's experiments shows that it is not a question of soil water supply, as this soil had an abundance of water. The unfavorable action of planting tomatoes on new ground which has recently supported growth of butternuts or walnuts also disposes of the first question, as the action on the plants occurs although the trees have been removed and could cause no depletion of the soil water. The situations of several trees closely observed by the writer disproved the probability of there being injurious substances formed through the decay of leaves and nut shells. These trees were so situated that strong winds prevented the accumulation of leaves and the accumulation of nut shells was not sufficient to cause any trouble. Were the second question to be answered in the affirmative, one would expect a more general and even dying of the plants over the area,

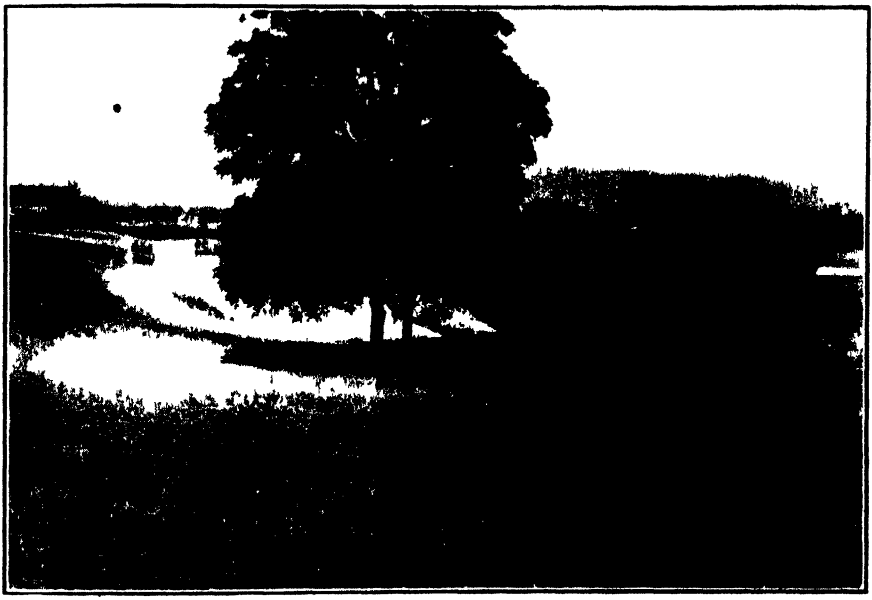


FIG. 2. Walnut trees in an alfalfa field, showing area in which alfalfa has been killed.

which is not always the case as will be shown later. The third explanation held the attention of the writer throughout the investigation. Do the roots of species of *Juglans* secrete a toxic substance which is injurious to some plants within their reach? To study this question, some walnut associations were examined critically and several experiments carried out.

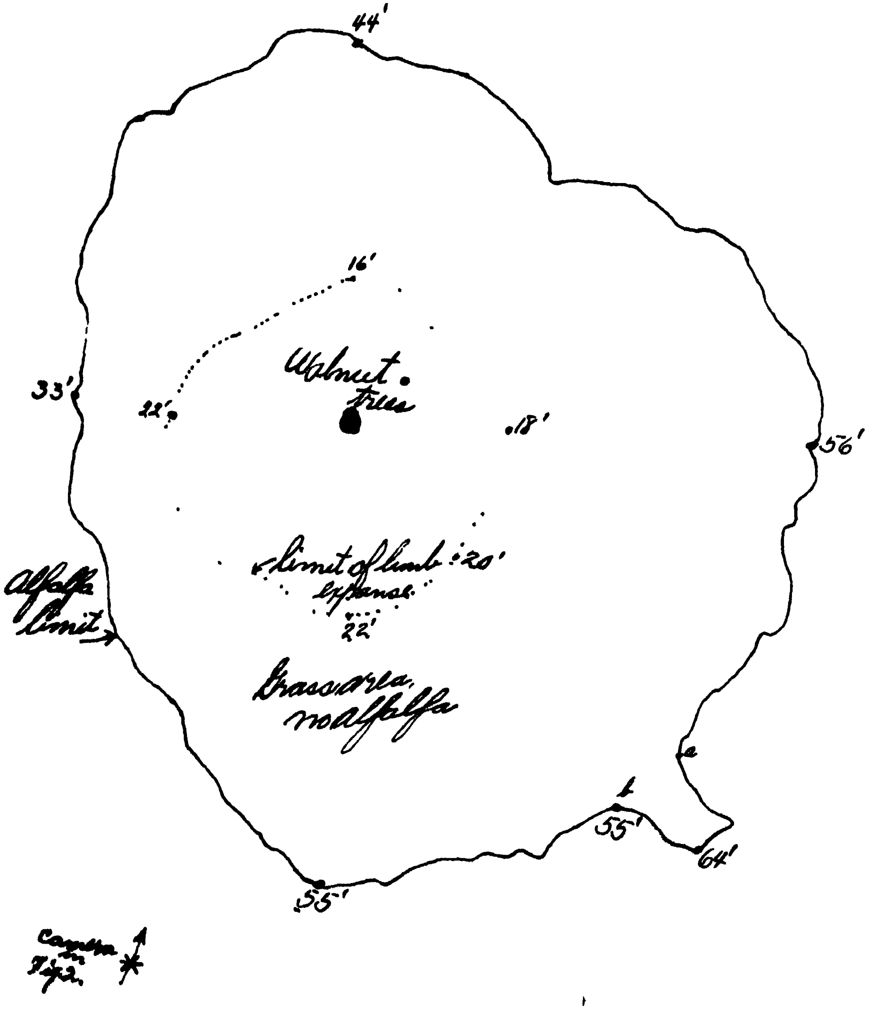


FIG. 3. Diagram of walnut trees shown in figure 2, indicating the position of the trees, limb expanse and area in which the alfalfa has been killed. Outside of area bound by the solid irregular line the alfalfa was green and healthy. Figures indicate distance from trunk of larger tree, given in feet.

WALNUT-ALFALFA ASSOCIATION

In a large alfalfa field of the Virginia Polytechnic Institute farm, there are two black walnut trees. One of these trees is of considerable size; the second is half dead through the work of borers. Around these trees it was observed that the alfalfa had been completely replaced by grass, as indicated in figure 2. The light area is entirely free from alfalfa and consists largely of timothy. Figure 3 is a diagram of the healthy tree and the border of living alfalfa plants. The limit of the limb expanse is shown by the dotted line, and the limit of the alfalfa-free area is shown by the irregular solid line. The extent of this area at first seemed to be too great in some directions to be equal to the expanse of the roots under ground; however, upon close examination of the first foot of soil at various intervals, it was found that walnut roots in every case reached to the extreme edge of the alfalfa-free area. At one edge of this area there was a marked extension, the end of which was 64 feet from the base of the walnut tree and 9 feet from the general border of the alfalfa-free area. This area was dug carefully to find whether walnut roots could be found within it. A trench was dug from point a to b, and a living walnut root three-fourths of an inch in diameter was found. The continuation of this root was such as to cause the irregular shape of this little area. The offshoot to the left was found to coincide with the development of a small end of the root underground. In other words, as the root came in contact with the alfalfa roots, the latter were killed. This case shows very strikingly that walnut trees are very injurious to alfalfa and that the dying of the plants is very closely associated with the development of the roots of the tree. There seems to be no general diffusion of any injurious substances through the soil; the action is always in the immediate vicinity of a walnut root. From this diagram one can also see that the dying of alfalfa could not be caused by shade; neither could it be caused by the decay of leaves, since the area is too large for the leaves of the trees to cover sufficiently to cause injurious action.

ASSOCIATION OF WALNUT WITH CERTAIN PLANTS IN HOME GARDENS

Four rows of tomatoes were planted specifically to study the action of the walnut on tomato plants. In the center of a general garden, four rows of tomatoes were planted in the vicinity of a walnut tree, as shown in figure 4. The tomatoes were set out on May 20. In the latter part of June, plants began to wilt and die. The wilting of the tomato plants was uneven over the area, and often one or two branches on the side of a plant became permanently wilted while the rest of the plant was healthy. Upon carefully removing the soil to expose the situation of the roots, it was found

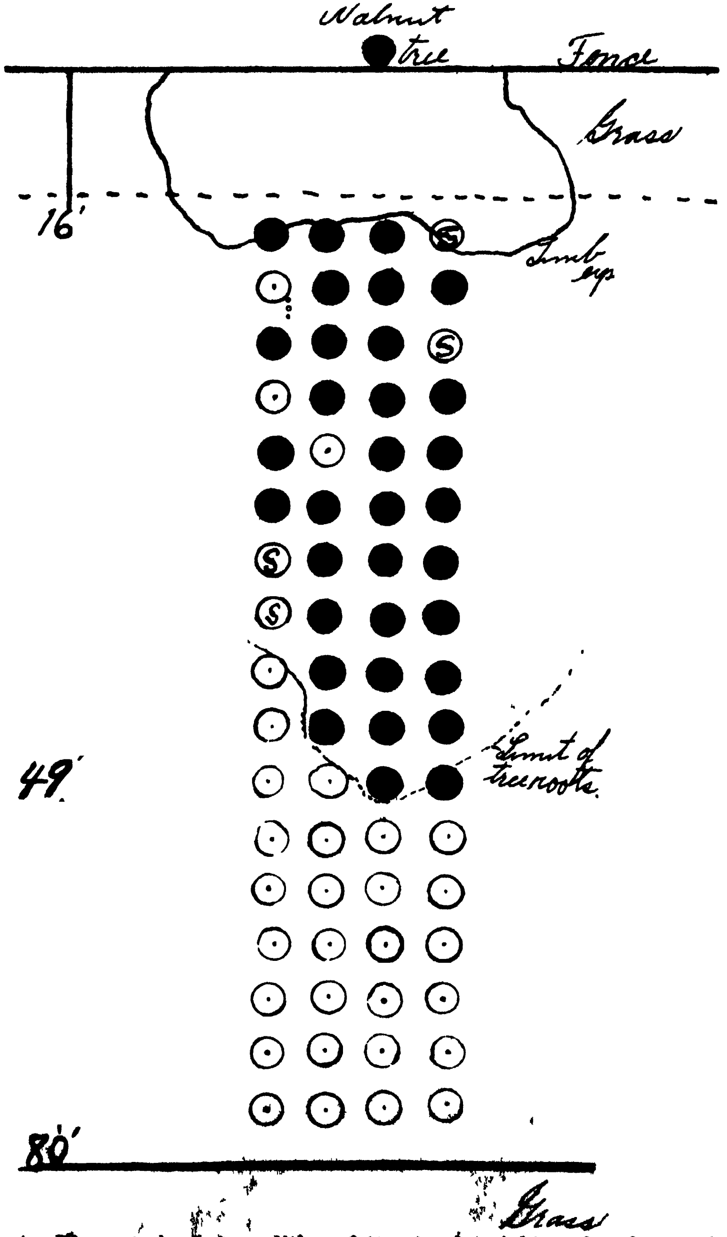


FIG. 4. Diagram showing condition of tomato plat eight weeks after setting plants in the immediate vicinity of a black walnut tree. Each circle indicates position in which a plant was set. White circles indicate plants which remained healthy. Circles with S in them represent plants that died soon after transplanting. Black circles indicate plants that wilted and died.

that in every case there was close contact between the tomato roots and those of the walnut. The plants wilted in a line sometimes diagonally across a patch, and sometimes parallel with the rows of tomatoes. This, it was found, was governed by the position of the walnut roots. In cases where one part of the plant wilted and the rest of the plant remained healthy, it was found that a walnut root ran between the rows or to one side of the plant and that one root came in close contact with those of the walnut, this one root always arising from the side of the plant on which the wilted branch was located. When the other portion of the plant began to wilt, it was found that roots from that side of the plant also had come in close touch with walnut roots. The direction of the roots underground could be traced without removing any dirt, by observing the development of wilt in the tomato plants. The probable distribution of the roots was predicted and later verified by removal of the soil. The presence of three unaffected plants within the affected area, marked by clear circles in figure 4, was at first not understood. They were well within the affected area and showed no signs of wilting. However, after digging them it was found that the roots of these plants did not come in contact with the roots of the walnut. The old walnut roots seemed to have as much effect on wilting of the plants as the young, actively growing ones. There was no specific relationship between the region of the strongest activity of the walnut roots and the wilting of the tomatoes, as would be expected if the trouble was due to lowering of the soil moisture.

On the right of the tomato patch, diagrammed in figure 4, irish potatoes were growing. There was a marked effect of the walnut on these potato plants, although it was not so distinct as on the tomatoes. Within the walnut-root invaded area of the garden there were also growing, in good condition, beets, snap beans, and corn, none of which showed any signs of wilting.

To determine whether the action on the tomatoes was one of toxicity from the walnut root, several pieces of bark from walnut root were placed in a water culture of tomato plant (fig. 5). Within 48 hours, plants in the water culture containing pieces of the bark were wilted and their roots browned, while the check plants in a similar solution with no walnut bark were erect and in good condition, thus indicating that there is some substance in the bark which is detrimental to the development of the tomato. In another experiment, large tubs of more than a bushel capacity were partly filled with soil and several pieces of walnut bark placed on the soil. More soil was added and young tomatoes transplanted to the tub. In four such tubs the tomatoes grew poorly, though they did not die. In two other tubs containing the same type of soil but no walnut root bark, the tomatoes grew normally. In a third experiment, soil was removed from the area in which the tomatoes shown in figure 4 were planted. This soil was taken at different intervals from the trunk of the tree and placed in separate large tubs. The intervals were 10, 12, 25, 50 and 80 feet from the trunk of the tree. None of the tomatoes planted in these tubs showed any evidence of toxicity from the soil, and one would conclude that there is no toxic substance generally distributed in the soil around walnut trees but that it is localized within the vicinity of the walnut roots.

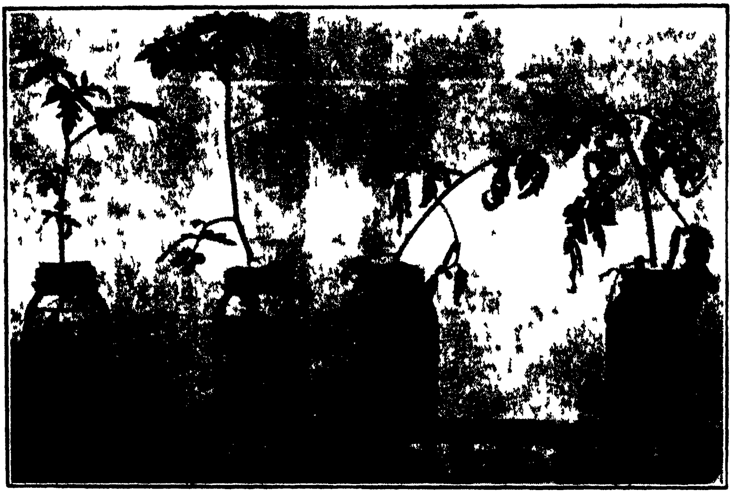


FIG. 5. Wilting of tomato plants in water culture. The two cultures to the left are checks in which no walnut bark was placed. The two jars showing wilting plants contained the same solution as the checks but with walnut root bark added.

Observations and experiments here reported indicate clearly that there is an antogonistic action of walnut roots which proves fatal to some plants. The action is evidently one of toxictiy. However, there is little or no poisoning of the soil as the roots of the affected plants must always be in close contact with those of the walnut. The toxicity is quickly apparent in water cultures of tomatoes containing walnut root bark, and slower in development where walnut root bark is incorporated in soil. The roots of the affected plant become brown in color and die. The tops die, not through direct action but because the roots cease to function.

The toxic principle, it would seem, is either insoluble in the soil water or it undergoes some chemical change shortly after leaving the walnut root, thereby losing its toxicity. Inasmuch as the roots of the affected plant are always in close contact with those of the walnut, there may be no secretion of the toxic principle by the walnut but it may be taken from the walnut by the action of the other roots.

Considering the constituents of the walnuts that may give rise to the toxic action, the substance juglone seems to be the most likely one, inasmuch as it is a naphthaquinone.

The quinones are known to have marked physiological action. Benzoquinone as a bacteriocide is 160 times more efficient than phenol in its action against *Bacillus typhosus,* (6). In a preliminary experiment containing two controls, the writer has found tomato cuttings in distilled water to be injured by benzoquinone in a concentration of 10 parts per million. Schreiner and Reed (4), in discussing the toxic action of several organic compounds upon wheat, state:

> "Chinone (Benzoquinone) is one of the most toxic compounds whose effect upon wheat seedlings was studied. A concentration of 100 ppm. was fatal in nine days and even 1 ppm. produced an injurious effect. In the intermediate concentrations the chinone was correspondingly injurious. The plants scarcely survived in a concentration of 50 and 25 ppm., and only in concentrations of 10 and 1 ppm. was there any growth comparable to that of the controls in distilled water.
>
> "The great toxicity of chinone is probably due to two of its chemical properties. The first of these is its strong oxidizing power, by virtue of which it is probably able to oxidize labile compounds which exist in cells of the plants and render them unsuitable for use in metabolism. The second property which gives chinone a toxic action is its ketone nature. Chinone readily forms bromin addition products. It united with one molecule of hydroxylamin to form chinon-oxime; with two molecules of hydroxylamin to form chinondioxime. The ketones, as is well known, are distinctly toxic to plants, and taken together these two properties undoubtedly account for the action of chinone upon plants."

The chemical properties of juglone are very similar; it forms mono- and dioxime and bromin addition products (7). It is an irritant causing violent sneezing and has been found, in comparison with benzo-quinone, to be especially valuable as a medicant for skin diseases. It is very slightly soluble in water but more so in organic acids. In dilute alkalin solution it undergoes oxidation.

On account of the difficulty of obtaining the pure substance, no experiments have been carried out to study the effect of this material on plants. It is hoped to do this in the near future.

SUMMARY

1. Walnut (*Juglans nigra* and *Juglans cinerea*) has an antagonistic action which causes a wilting and dying of certain plants such as alfalfa, tomato, and potato.

2. Roots of the affected plants were always in close contact with walnut roots; the toxic substance is not generally distributed in the soil around walnut trees, but is localized in the vicinity of the walnut roots.
3. Walnut root bark contains a substance which is toxic to the roots of tomato plants grown in water culture.
4. It is likely that juglone, or some similar substance, is the toxic constituent of the walnut.

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BLACKSBURG, VIRGINIA

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PHYSIOLOGIC SPECIALIZATION AND MUTATION IN HELMINTHOSPORIUM SATIVUM[1]

J. J. CHRISTENSEN[2]

WITH FOUR FIGURES IN THE TEXT

INTRODUCTION

Helminthosporium sativum P. K. and B. has been studied extensively in recent years. It is an extremely interesting and important pathogene. It causes seedling blight, root rots, basal stem rot, spike and seed blight, leaf spots, stem lesions, and premature death of wheat, barley, rye and many grasses. Although the pathogene has been investigated extensively, there have been many conflicting statements regarding its morphological and physiological characters and its pathogenicity. These conflicting results can be explained very largely by the fact that *H. sativum* is a group species of many physiological forms which mutate readily. The writer has studied thirty-seven forms in detail. A knowledge of the number, cultural characteristics, parasitic capabilities, and genetic stability of these forms is prerequisite to a proper understanding of their pathogenicity and is essential to the plant breeder in his endeavor to develop resistant varieties of grain. Just as important is it to know how frequently these forms mutate, the behavior of the mutants in culture, and their effect on the host.

OBJECTS OF THE INVESTIGATION

The objects of the investigation were: (1) to ascertain the number of physiologic forms; (2) to ascertain the differences in virulence or pathogenicity of the different forms and their mutants; and (3) to determine the degree of stability of these forms.

METHODS AND MATERIALS

All physiologic forms used in the following studies were derived from single spore isolations. The method of procedure was as follows: A mass of spores was taken from the culture with a platinum wire and mixed with 15–20 cc. of sterile water in a test tube. About 0.5 cc. of this spore suspension was poured into a tube of melted agar. Usually two more successive cultures were made in a similar manner. The agar from each tube was

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[2] The writer gratefully acknowledges the helpful advice and criticism of Dr. E. C. Stakman, under whose direction the work was done.

poured into a sterile petri dish, 10 cm. in diameter, and incubated at room temperature. From 12 to 24 hours after the spores had germinated, the plates were examined. Germinating spores, sufficiently far apart, were marked by a circle of India ink on the petri dish. The marked area was carefully examined as to purity from germinating bits of mycelium and germ tubes of other spores before they were cut out and put into separate tubes of agar. The piece of agar containing the germinating spore was first placed on the side of the tube and examined to ensure that only one spore was introduced into the new medium.

The seeds of cereals were obtained from the United States Department of Agriculture and from the Agronomy Division, University of Minnesota. All seeds used in pathogenicity experiments were treated with Jensen's modified hot water treatment or with Chlorophol, an organic mercury compound, in order to kill, in so far as possible, intraseminal mycelium.

The soil used in the experiments was a mixture of three parts garden loam and two parts sand. All the soil for a given experiment was mixed in one lot and then steamed for three hours. The inoculum was grown in Erlenmeyer flasks on autoclaved seed of wheat and oats, in a proportion of three to one by volume. Even the forms that grew slowly on ordinary culture media grew well on this medium. An equal amount of inoculum was added to each pot of a series. The same quantity of uninoculated wheat and oats mixture was added to the controls. The pots were placed on a center bench in the greenhouse in order that all might receive the same amount of heat and light.

EXPERIMENTAL RESULTS

Cultural Characteristics. More than fifty forms were studied, thirty-seven of them in detail. In order to compare their cultural characters, the thirty-seven forms were grown on two different media: first, a one per cent potato dextrose agar; second, a mixture of oatmeal, rice, and cornmeal. Some forms were grown on other media also. Triplicate plates of each medium were inoculated with each form. Small, and as nearly as possible equal, portions of medium containing mycelium, and usually spores, were used as inoculum in each case. The plates were of uniform size and contained the same amount of medium, usually 18 cubic centimeters. The agar was made in one lot, tubed, sterilized, and poured at the same time. After inoculation the plates were placed on the same table in the laboratory and kept at room temperature. Thus all were subjected to the same general environmental conditions. The triplicate colonies of the same forms on the same medium were always alike, unless contaminated by other organisms. It was noticed that a small colony of bacteria sometimes profoundly affected the growth of certain forms. In the immediate vicinity of colonies of a foreign organism, sporulation was often stimulated, and the morphology of the mycelium was occasionally changed considerably.

The thirty-seven forms studied can be differentiated on culture media macroscopically by the following characters: rate of radial growth; relative amounts of submerged and aerial mycelium; nature of mycelial growth, whether woolly, or cottony, etc.; zonation, whether lacking, prominent, moderate, or faint, and frequence of zones and distance apart; conidial production, whether absent, scarce, moderate, or abundant; conidial clusters; and color of mycelium, white to black with intervening gradation and tints of other colors. The length, width, shape, and septation of the conidia also are different in some of the forms.

The cultural characters of many of the forms of *H. sativum* differ greatly from each other on the same medium. The cultural characters of the same forms are also different on different media. The colonies of many forms were so strikingly different in general appearance on a given medium that one was prone to separate them into different species. However, their similarity on other media forbade such a reclassification.

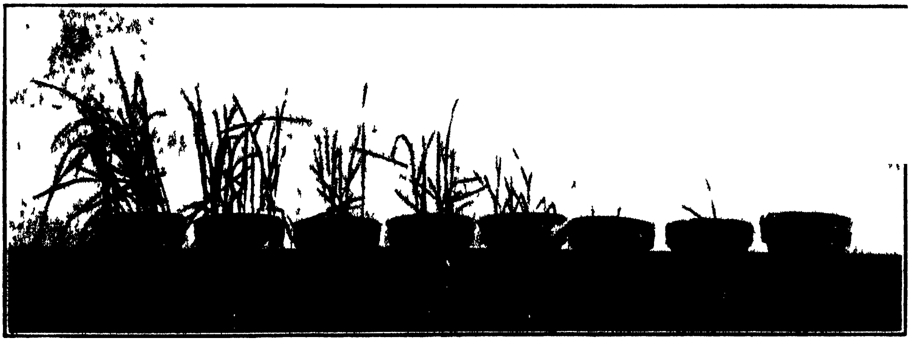

FIG. 1. Seedlings of Marquis wheat grown in soil inoculated at time of planting with *H. sativum*, showing the comparative virulence of different forms of the pathogene.

A Control
B Form 26
C Form 21
D Form 3
E Form 19
F Form 22
G Form 8
H Form 5

Pathogenicity of Physiologic Forms. Christensen (5), in 1921, noted variations in the degree of pathogenicity of certain physiologic forms of *H. sativum*. Dosdall (6) found a difference in degree of severity with which two strains of *H. sativum* attacked Lion barley and Marquis wheat. Henry (8) observed variation in virulence of strains of *H. sativum* obtained from different sources and was able to distinguish four strains of "small-spored Helminthosporia" by their differences in pathogenicity on wheat.

The writer made tests of the comparative virulence of physiologic forms by inoculating soil in four 4-inch pots with pure cultures. Twenty-five seeds were planted in each pot, so that one hundred seeds of each cereal variety were used in each test.

The comparative pathogenicity of twenty-six forms was determined on Marquis wheat (C. I. 3641) and on Mindum (Minn. No. 470), a durum variety. Both varieties were more or less susceptible to all twenty-six forms of the pathogene, but there were distinct and consistent differences in the virulence of different forms on both varieties of wheat. Forms 3, 19, 21, and 26 were relatively weak (Fig. 1). There also were intermediates between the two extremes.

Differences also exist in the virulence of the different forms on varieties of barley. Trebi (C. I. 936) and Chevalier (C. I. 278) were inoculated with all the thirty-seven forms. Many of the physiologic forms differ in their reaction on two varieties of barley. Forms 5, 11, 34, and 37 were especially virulent on both varieties. Two of these forms, 5 and 11, were virulent on wheat also. Although Forms 3, 4, 30 and others were weakly parasitic, they all attacked barley slightly. In general, the same forms were also weak pathogenes on wheat varieties.

Stability of the Physiologic Forms. The range of variability of *H. sativum* is very wide. The character and rate of growth, the ability to reproduce, and the morphology of the organism are influenced profoundly by the kind, proportion, and amount of foods available and by other environmental conditions. Dosdall and Christensen (7), Stevens (9), and others have demonstrated this conclusively. Besides, it has been shown that there are many forms of *H. sativum* which respond differently to various ecological conditions.

Temporary phenotypes, or modifications, induced by differences in environmental conditions, occur frequently, although the genotype remains unaltered. Such variants revert to the parental phenotype as soon as the causal stimulus is removed. Comparative cultural tests carried on for several years indicate that some forms of *H. sativum* remain constant and always appear the same under identical conditions. The question then arises, how did the forms originate and are they relatively stable? New forms of life are generally supposed to arise as a result of hybridization or mutation.

Recent publications (1, 2, and 9) indicate that asexual mutations in fungi occur frequently on culture media. In 1922, Stevens (9) presented evidence to show that mutations were common in *Helminthosporium* growing in culture. In the present studies much of Stevens' work was repeated and extended, and in general the results were corroborated.

Numerous and widely different types of mutants, occurring usually in sectors, have been observed repeatedly. These variations arose from parts of mycelium and were generally wedge-shaped or fan-like in shape (Figs. 2 and 3). Under certain conditions, mass-like mutation apparently occurred in some forms. This type of mutation recently has been described also in higher plants (3).

In the present work, numerous transfers were made from sectors and normal parent material. When the original variant was chosen from a sharply defined sector of a relatively young culture, as illustrated in Fig. 2, it always developed into a colony distinct from its parent. Furthermore, a study of subsequent transfers from these sectors indicated that the changes were genotypic and not merely changes in phenotypes due to environmental conditions. Single-spore isolations, whether from the sector in which a variant originated, or from colonies developed from mass transfers, made similar growth on culture media. In either case the colonies were all alike. Mutants bred true when propagated from spores or mycelium. Stevens (9), Burger (4), and others also have demonstrated that spores from mutants again produced the mutant type.

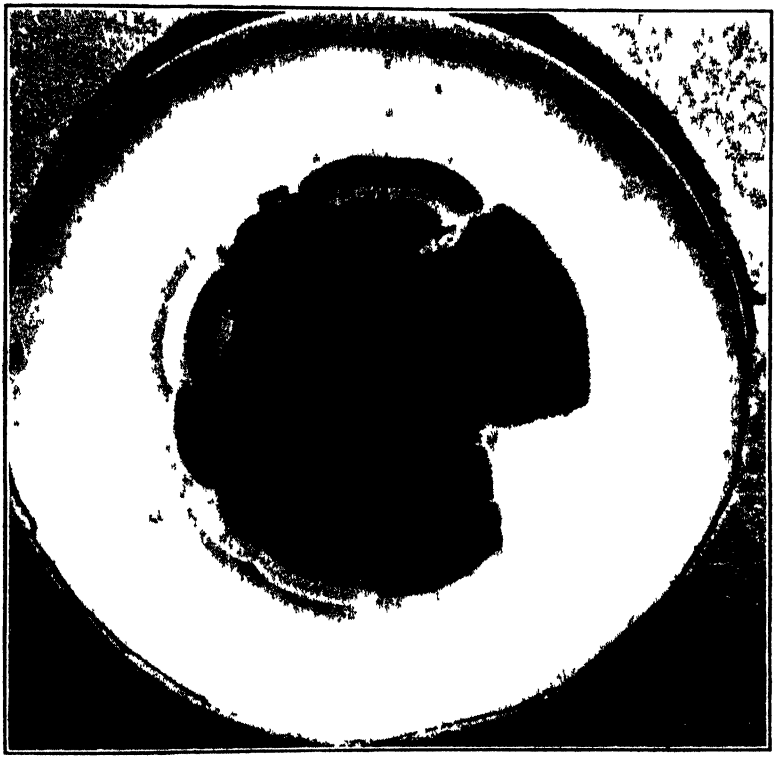

FIG. 2. *H sativum,* Form 1, on oatmeal rice cornmeal agar, showing mutants.

In order to ascertain whether there were any differences in the tendency for various forms to mutate, two kinds of media were inoculated. Each form was grown in triplicate plates of uniform size, containing 18 cc. of the medium. The plates for each series were inoculated on the same date and kept on the same table in the laboratory.

Some forms mutate more often than others. A given form may mutate more frequently on one nutrient medium than on another. On potato dextrose agar, eighteen out of thirty-seven forms developed sectors or apparent mutations, while sectors occurred in cultures of twenty-seven of the thirty-seven forms when grown on oatmeal-rice-cornmeal agar. Seven forms failed to develop sectors on either medium. Forms 26, 29, and 37 gave rise to apparent mutations on potato dextrose agar but none on the other medium. Twelve of the forms which did not mutate on potato dextrose agar did so on the oatmeal-rice-cornmeal agar. The differences in the number of sectors of certain forms on the two media were sometimes very marked. Thus, Form 33 developed thirty-six; Form 30, fifteen; Form 16, eight sectors on oatmeal-rice-cornmeal agar but on potato dextrose agar none, five, and one, respectively. Mutations occurred in forms obtained from England, Australia, Africa, Argentina, Serbia, and Canada, and from various localities in the United States.

Some of these mutants have been grown on several different media for varying lengths of time, but they have remained permanent for the characters under observation. Mutants appeared as stable as the various forms of *H. sativum* which have been isolated from naturally infected plants. Although it is true that several mutants apparently reverted, these reversions were always in the form of a sector. This agrees with the observations of Stevens (9). Temporary modifications, such as sometimes appear—especially in old cultures—and which revert to the parental type on the first transfer, are not considered here.

Some of the mutants which were cultured mutated still further. Colonies of a mutant have been observed to produce distinct sectors which in turn mutated while still in the same plate. Two mutants gave rise constantly to new mutants, their action suggesting genetic contamination.

In order to determine the effect of the amount of medium on the expression of variation, the following test was made. Newly poured agar plates were placed on a slight incline so that the upper side was covered by a thin layer of the medium, the lower by a considerably thicker layer. These slants were inoculated with Form 1 in the center in the usual manner. Numerous sectors developed, but they developed only in the region of the thinner layer of agar (Fig. 3). That at least some of these sectors were pure mutants was proved by successive transfers.

The mutants studied varied from the parents in the following characters: (1) rate of growth, (2) nature of growth, (3) conidial production, (4) conidial clusters, (5) mycelial characters, (6) color, and (7) zonation. Many of the gross characters in some forms were quite different from any of those observed in strains or forms isolated from host plants. These mutants differed from each other culturally quite as much as do certain species of *Helminthosporium*, such as *H teres* Sacc., *H. pedicellatum* Henry, and *H. gramineum* (Rab).

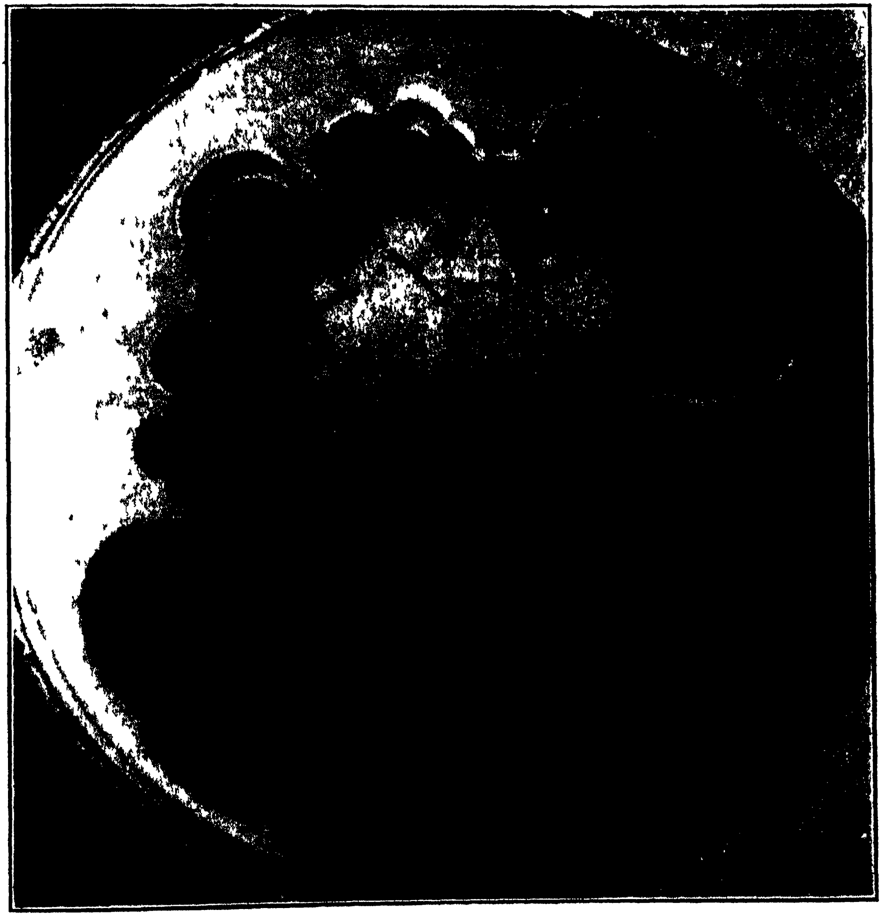

FIG. 3. *H. sativum*, Form 1, on oatmeal-rice-cornmeal agar slant placed so that the depth of agar at the bottom was about three times that at the top. Mutants only on the shallow half of the medium.

Apparently most mutations in fungi have their origin in the loss of a factor, or a group of factors, for color, and in the loss of a factor for high fructification. Stevens (9) states that mutants with low conidial production, verging on sterility, coupled with paleness of colony, occurred with the greatest frequency. This is in accord with the writer's findings.

Just how these mutations arose is not known. They may have arisen through what one might term normal nuclear rearrangement, or through aberrant chromosomal distribution, or gene changes. It has been impossible to determine accurately the genetic constitution of the parents and their mutants, because the nuclei and chromosomes are very small and the plants are propagated asexually, thus rendering cross-breeding experiments futile. Even if a variant resulted from the fusion of two adjoining cells, it might not necessarily be considered a normal process of combination or segregation, as it has been shown that anastomosing of hyphae is an extremely common phenomenon in *H. sativum.*

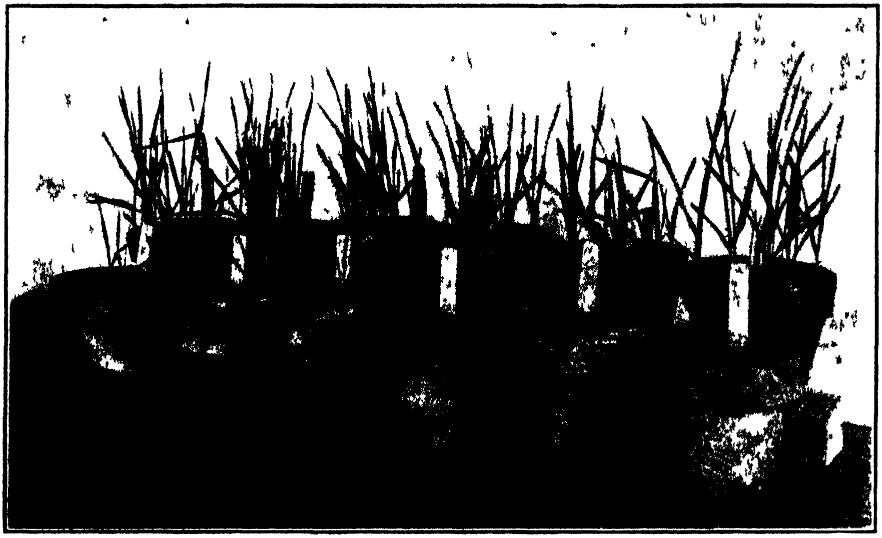

FIG. 4. Seedlings of Marquis wheat grown in soil inoculated with *H. sativum,* Form 22 (back row), and its mutant, No. 40 (front row), showing the difference in virulence of parent and mutant.

Pathogenicity of Mutants Compared with that of their Parents. In order to ascertain whether there are any differences in the degree of virulence of mutants and their parents, comparative tests were made on Trebi barley and Marquis wheat. The experiment was similar to that already described for testing the pathogenicity of the 37 forms of *H. sativum* on barley, except that six pots of each cereal were used. The virulence of thirteen mutants was compared to that of their parents.

All of the mutants did not possess the same degree of virulence. Most of them were like the parent form, but two were decidedly more virulent than their respective parents. Mutant No. 40 was outstandingly more virulent than its parent, on both barley and wheat (Fig. 4). Three of the mutants were less virulent than their parents.

Mutant No. 40 was derived from a single spore of a monosporous culture of Form 22. It arose as a distinct sector, similar to the one shown in fig. 2, and had been transferred several times and grown on three different nutrient media. The cultural characteristics were not only different from those of its parent, but were unlike those of any of the forms under observation.

DISCUSSION AND CONCLUSIONS

H. sativum is a group species consisting of many physiologic forms. At least thirty-seven can be recognized readily on culture media. The writer observed at least fifty distinct forms, and there are indications of the existence of numerous others. Eleven distinct forms were isolated from material collected in the vicinity of St. Paul. Forms obtained from other workers, or isolated from material collected in different regions, as a rule were different.

The thirty-seven forms studied by the writer differ from each other consistently under the same conditions, and each form is extremely variable under different conditions. For this reason, extreme caution is necessary in describing species such as *H. sativum* and other fungi which may vary to a like degree. At best, a description of *H. sativum* must be based on the characters of relatively few physiologic forms. Not only do the physiologic forms differ in general appearance on culture media, but they are strikingly different physiologically. What is most important, however, they differ pathogenically.

Many of the physiologic forms have quite different parasitic capabilities on wheat and barley. Some forms are extremely virulent, others are moderately virulent, and still others are but weak parasites. These differences in virulence of forms are of paramount importance, as they complicate the study of genetic inheritance and the development of resistant varieties. Varieties which are resistant in one region may be very susceptible in another. Therefore a study of the number and the parasitic capabilities of physiologic forms is prerequisite to sound procedure in breeding work. The problem is essentially local, or, at best, regional. Results obtained in one locality may not be applicable in another.

Mutations occur frequently on culture media. Some forms mutate much more often than others. The mutants observed differed from the parents in rate of growth, color, zonation, amount of aerial mycelium, and pathogenicity. Relatively weak forms may produce very virulent mutants and *vice versa*. This change in parasitism is tremendously important. *H. sativum* is dynamic, not static. It is continually changing, and consequently presents a continually changing problem. There is every reason to suppose that mutation occurs in nature as well as on artificial media. A final solution of the problem is therefore extremely difficult.

The nuclear phenomena involved in the mutation of *H. sativum* are not known. The possibility of segregation as a result of nuclear fusion is not excluded, although it does not seem to be very strong. Anastomosing hyphae have often been observed. It is barely possible that hybridization may occur quite frequently. The mutants, which arise as sectors, however, suggest that the mechanism is somewhat analogous to bud mutations in higher plants.

SUMMARY

1. There are numerous physiologic forms of *H. sativum*. The writer studied thirty-seven in detail.

2. The thirty-seven physiologic forms can be distinguished in culture by the following characters: rate of growth, relative amounts of submerged and aerial mycelium, nature of mycelial growth, zonation, production of conidia, density of conidial clusters, the color of the mycelium, and the readiness with which the forms mutate.

3. All forms can attack the roots and basal stems of wheat and barley Some forms are very virulent, while others are relatively non-virulent. In general, there is a correlation between the virulence on wheat and barley.

4 The differences in the pathogenicity of different forms are so great that the conflicting results obtained by previous investigators easily can be explained.

5. Asexual mutation occurs frequently in some forms of *H. sativum*. Mutants arise abundantly from some forms in artificial culture as wedge- or fan-shaped sectors. These mutants bred true when propagated from either spores or mycelium.

6. Some forms mutate readily, others do not. Mutation was observed in forms from England, Australia, Africa, Argentina, Serbia, Canada, and from many localities in the United States.

7. Apparently reversions occur, but, when they do, they are always in the form of sectors.

8. The mutants differ from their parents not only in morphological characters but also in pathogenicity. Some are more virulent than the parents, others are less so.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
ST. PAUL, MINNESOTA.

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STUDIES ON THE PATHOGENICITY AND PHYSIOLOGY OF HELMINTHOSPORIUM GRAMINEUM RAB.[1]

THORVALDUR JOHNSON

The barley stripe disease caused by *Helminthosporium gramineum* Rab. has been known for a long time. The pathogene is almost an obligate parasite, and there are practically no authentic records that it has produced conidia on artificial media. Furthermore, the disease has been interesting because the pathogene was supposed to infect plants at flowering time and to cause a systemic infection like that caused by the loose smut fungi. Certain doubts, however, have been raised regarding its exact life history. There seems to be some evidence that the seed or very young seedlings may be infected. There also has been a question as to why the disease should be distributed as it is. It occurs commonly in the more northern barley growing regions, but it is practically unknown in those farther south. The development of the disease differs also in different seasons. The explanation for this seasonal variation might be either differences in temperature or the existence of different physiologic forms. As many of these essential facts regarding *H. gramineum* were obscure, the writer undertook an investigation to elucidate some of the questions.

OBJECTS OF THE INVESTIGATION

The specific objects of the investigation were:

1. To determine the effect of temperature on the severity of infection.
2. To ascertain whether infection would result from inoculating seed or seedlings.
3. To find out whether there are physiologic forms.
4. To determine the conditions most favorable for growth and fructification.

THE RELATION OF TEMPERATURE TO INFECTION

It is well known that early-sown barley usually develops more stripe than that sown at a later date.

Ravn (6), in 1896, made field observations on barley grown from diseased seed planted on May 6 and May 19 respectively. That sown at the former date developed 37.7 per cent of diseased plants, while that sown later produced 21 per cent of infected plants. It therefore appeared likely

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[1] Published with the approval of the Director, as Paper No. 560 of the Journal Series of the Minnesota Agricultural Experiment Station.

that a relationship existed between temperature during germination and early growth and infection, but Ravn made no controlled experiments to determine this.

Therefore the writer conducted experiments to ascertain whether such a relationship existed. Seed of Minsturdi barley (Minn. 439), containing naturally infected kernels, was planted in six-inch flower pots (20 kernels per pot) which were placed in soil temperature tanks in the greenhouse at 18–20° C., 23° C., 27° C. and 32° C., eight pots being kept at each temperature. Four pots were kept at from 10–12° C. in a temperature tank regulated by running water. The soil in these pots was covered with a layer of ground cork which served as an insulator between the atmosphere and the soil.

The seedlings were of approximately equal development when removed from the tanks, except in the case of those at 10–12° C., which were smaller when removed. After their removal from the tanks, all the pots were kept under similar conditions in a moderately cool place in the greenhouse.

The first appearance of stripe was observed on January 31, exactly one month after planting, in plants kept at 10–12° C. The average height of the plants in the pots kept at that temperature was then six inches, while those kept at other temperatures were almost ten inches in height. The results are presented in table 1.

TABLE 1.—*The effect of soil temperature on the infection of Minsturdi barley, Minn. 439, by Helminthosporium gramineum*

Soil temp. in degrees C.	Time required for emergence of plants	Time kept in temp. tanks	Number of plants	Plants infected	
				No.	Per cent
10–12	13 days	15 days	75	9	12.
18–20	6 "	9 "	160	7	4.3
23	4 "	7 "	160	1	0.62
27	3 "	7 "	160	1	0.62
32	3 "	7 "	160	1	0.62

The results show: (1) that low temperatures favor infection; (2) that the critical period for infection is during, and immediately after, seed germination.

These results furnish evidence to explain the occurrence of the disease in certain seasons, and its distribution throughout the barley-growing regions of the world. Temperature undoubtedly explains the comparative immunity of barley in warm regions and the severity of the disease in the cooler climates.

SEEDLING INFECTION BY H. GRAMINEUM

Various considerations have led the writer to think that *H. gramineum* might infect seedlings more frequently than is commonly supposed.

Tisdale (8) has shown that *Ustilago tritici* and *U. nuda* may commonly infect seedlings of wheat and barley respectively. Some of the more recent work on *H. gramineum* also lends support to this view. Smith (7), in England, as a result of a re-investigation of the life history of *H. gramineum,* came to the conclusion that Ravn (6) was mistaken in believing that the fungus inhabited the growing point of the host. Smith believes that infection occurs while the shoot is still under the adherent glumes, or during its emergence. The inoculum comprises (a) conidia lodging at the awn end of the grain; (b) mycelium penetrating from the glumes; and (c) perithecia formed inside or outside the glumes. He thinks that the coleoptile, and not the embryo, becomes infected.

Vogt (9) in Germany, in 1923, came to similar conclusions. Histological studies led him to conclude that the mycelium inhabits the space between the glumes and the pericarp of the seed but is never found either in the embryo or the endosperm, and that infection takes place through the coleoptile rather than through the embryo.

Since the time of Von Post, *H. gramineum* has generally been considered to cause "floral infection." While this term definitely limits the time of infection, it does not define the manner of infection nor the tissues of the kernel attacked. In fact, the only thing which the term signifies is that the pathogene is seed-borne. It has been commonly supposed that seedling infection either does not occur or plays only an insignificant part in the life history of the pathogene. Ravn (6) attempted to determine the relative frequency with which the pathogene could attack germinating seedlings. Two hundred and fifty dehulled barley grains of susceptible varieties were germinated in contact with mycelium, with the result that ten plants (4 per cent) became infected. From this Ravn concludes "that stripe disease may be produced by infecting the embryo." He adds, however, that the principal method of infection is by conidia which cause infection during flowering time, forming a mycelium within the seed where "the fungus remains latent with the embryo so long as the grain is dry, and revives when germination commences."

The writer conducted experiments to determine to what extent *H. gramineum* could infect germinating seed. Seed of the susceptible variety Minsturdi (Minn. 439) was used. Ordinary seed and dehulled seed were both inoculated during germination, in various ways, with spores and mycelium of the pathogene. All seed was previously treated by Jensen's hot water treatment, either at 52° C. for 15 minutes or at 46–48° C. for two hours, in order to kill any mycelium in the seed. Seeds treated with mycelium and spores were sown in six-inch pots kept in temperature tanks at from 18–20° C. The first indications of stripe disease were seen three weeks after planting. Table 2 summarizes the results of the first experiment.

TABLE 2.—*The results of inoculating normal and dehulled seed of Minsturdi barley, Minn. 439, with mycelium and spores of Helminthosporium gramineum*

Kind of seed	Previous hot water treatment	Inoculum	Total number of plants	Plants infected	
				Number	Per cent
Dehulled	52° C. for 15 min.	Spores	44	5	11.3
		Mycelium	34	8	23.5
		Check	20	0	0
Normal	52° C. for 15 min.	Spores	40	0	0
		Mycelium	40	1	2.5
		Check	20	0	0
Normal	46°–48° C for 2 hours	Spores	40	0	0
		Mycelium	40	0	0
		Check	20	0	0

It is evident from table 2: (a) that infection may occur during germination of the seed; and (b) that the period of greatest susceptibility is prior to the emergence of the coleoptile from the glumes.

Variations in the amount of infection in different experiments may be ascribed to varying technique, as various methods of applying the inoculum were tried.

In the next experiment all seeds were dehulled and soaked in hot water at 52° C. for 15 minutes. Those inoculated with spores were soaked in a spore suspension for a few minutes; others were mixed with mycelium on agar and the soil was also inoculated with mycelium at the time of planting. One-half of the pots were kept at 16° C., the others at 22° C. Table 3 summarizes the results of this experiment.

The first infection was observed nine days after sowing, when the normal seedlings were about four or five inches high. The affected seedlings varied in height from one-half inch to three inches. Most of these early infections resulted in the death of the plants.

An attempt was made to determine the amount of artificial infection which could be induced by inoculating germinating seeds from which the hulls were not removed. Seed was treated in hot water as above at both 46–48° C. and 52° C. Spores and mycelium were both used as inoculum. A spore suspension was poured into melted agar which had been cooled to below 50° C. The seeds were coated with this agar and germinated between moist blotting papers and finally planted in sterilized soil in six-inch pots. Other seeds were germinated in contact with mycelium on agar in petri dishes and were then planted as above and placed at greenhouse temperatures. The results are summarized in table 4.

TABLE 3.—*Results of inoculating dehulled seeds of Minsturdi barley, Minn. 439, with Helminthosporium gramineum*

Temperature	Inoculum	No. of plants	No. of plants infected	Per cent of plants infected
16° C.	Spores	45	10	22.2
	Mycelium	45	34	75.5
	Check	25	1	4.0
22° C.	Spores	50	16	32.0
	Mycelium	39	23	58.9
	Check	25	0	0

The results were not as conclusive as these figures indicate, because soaking in water at 46–48° C. did not kill all the hyphae in naturally infected seed. Further, the number of seedlings from seed treated with mycelium was reduced, either through the action of the mycelium or the severity of the hot water treatment at 52° C.

TABLE 4.—*Results of inoculating germinating normal seed of Minsturdi barley, Minn. 439, with Helminthosporium gramineum*

Previous hot water treatment	Inoculum	No. of plants	No. of plants infected	Per cent of plants infected
46–48° C. for 2 hrs.	Spores	35	3	8.5
	Mycelium	33	8	24.2
	Check	33	3	9.0
52° C. for 15 mins.	Spores	40	9	22.5
	Mycelium	9	4	44.4
	Check	20	0	0

At any rate, the results indicate that infection of susceptible hulled varieties is possible by inoculating the seed, even though the hulls are not removed. However, it appears more difficult to obtain infection when the hulls are left on the seed than when they are removed.

There has been some difference of opinion among investigators as to the manner in which infection takes place. Ravn (6) states that normally the embryo is infected, and he claims to have histological evidence for the presence of hyphae in that organ. Vogt (9), as the result of histological studies, claims that neither embryo nor endosperm is infected but that the mycelium is located principally between the glumes and the pericarp of the seed. This view is supported by Smith (7) who is of the opinion that the disease is not truly systemic, as held by Ravn and others.

The fact that it is possible to produce the disease by inoculating germinating seed from which the glumes have not been removed shows that infection may take place through the coleoptile, as the embryo is naturally protected in such seeds. Further work probably is needed to settle definitely the question of the manner of normal infection.

From the point of view of the investigator, the practical value of coleoptile infection by *H. gramineum* is twofold: (1) it affords a means of studying varietal resistance of barley; and (2) it may be used for studying physiologic specialization of the pathogene.

PHYSIOLOGIC SPECIALIZATION

In the light of what Christensen (1) and others have found regarding *H. sativum* P. K. and B, it would not be surprising to find physiologic forms of *H. gramineum*.

A considerable number of single spore isolations were made from material collected at various localities in Minnesota, Colorado, and in Saskatchewan and Alberta, Canada. In investigating the effect of temperature on the growth of the cultures from these isolations, it was found that when they were kept at 32–33° C. for from ten to twelve days and then removed to room temperatures, all the cultures except one recovered and grew normally. The culture in question was obtained from a single spore isolation from infected barley collected at Edmonton, Alberta, Canada. The experiment was repeated several times and similar results were obtained. Evidently this strain is less resistant to high temperatures than the others.

To determine whether the culture was *H. gramineum,* dehulled barley seed was inoculated, and typical stripe disease lesions appeared on the plants.

Morphologically, the mycelium of this strain is not distinguishable from that of the others, nor was any appreciable difference observed in the optimum temperatures of this and other strains. The minimum temperature permitting growth was not determined. The Edmonton strain, however, grew slightly better at from 5 to 6° C. than the others, and it seems clear that its temperature requirements are different from those of the other strains, indicating that it is a distinct physiologic form.

FACTORS INFLUENCING GROWTH AND REPRODUCTION

Helminthosporium gramineum does not readily fructify on artificial media, in this respect differing from other species of *Helminthosporium* which cause cereal diseases. As far as the writer knows, Paxton (5) is the only worker who has reported the production of conidia of *H. gramineum* on artificial media. He obtained perithecia on barley straw and transferred the ascospores to cornmeal agar on which conidia were produced.

The writer attempted to determine the factors influencing the sporulation of *H. gramineum* on artificial media. Several strains were tested, but most of the work was done with a single strain. Persistent attempts to find the right combination of factors for sporulation were made for about seven months, but they were unsuccessful. As the results were negative, it does not seem worth while to discuss the experiment in detail.

The effects of temperature, light, moisture, aeration, hydrogen-ion concentration, various types of media, both solid and liquid, plant tissue extracts, yeast extracts, and numerous variations of nutrients, were tried. The addition of a number of other fungi, and of bacteria, to the culture was also tried with negative results. The addition of certain bacteria resulted in the production of spore-like bodies which, however, failed to germinate. Similarly, the sudden removal of the nutrient solution, coupled with constant light, induced the production of abnormal structures and spore-like bodies, but no normal conidia were formed. On the other hand, variations in aeration, humidity, hydrogen-ion concentration and amount of nutrients resulted only in different degrees of quantitative growth.

In carrying out these experiments, close attention was paid to previous work along similar lines by Klebs (3), Coons (2), Leonian (4) and others, but invariably the methods which they had used successfully with other fungi failed to produce similar results with *H. gramineum.*

The maximum hydrogen-ion concentration which permitted growth was determined as pH 2.52. The limits of hydroxyl-ion concentration were not determined but lie beyond pH 9.25.

The maximum and minimum nutrient concentrations for growth were determined with respect to a number of sugar solutions and found to be respectively $\frac{M}{1}$ and $\frac{M}{1000}$, while optimum growth occurred at from $\frac{M}{10}$ to $\frac{M}{100}$.

SUMMARY

Low soil temperatures were found to favor infection of barley by *H. gramineum* Rab. under controlled experimental conditions. The greatest infection occurred at from 10° C. to 12° C. Very little infection occurred at soil temperatures higher than 20° C.

It has been shown that artificial infection by *H. gramineum*, through inoculations of germinating barley seed, is possible. By removing the hulls it is possible to obtain a high percentage of infection in susceptible varieties. A lower percentage of infection occurs when normal seed of the same varieties is inoculated. The period of greatest susceptibility in dehulled seed appears to be just subsequent to the emergence of the coleoptile.

Evidence has been obtained for physiologic specialization in *H. gramineum*. At least two physiologic forms appear to exist.

Attempts to induce this organism to produce conidia on artificial media were unsuccessful.

The writer wishes to express his appreciation of many helpful suggestions from Dr. E. C. Stakman and Dr. J. J. Christensen. He also wishes to express his gratitude to Dr. Margaret Newton and Prof. W. P. Fraser, of Saskatoon, Sask., Canada, for help in collecting material.

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SECOND REPORT OF PROGRESS ON STUDIES OF CROWNGALL IN RELATION TO NURSERY STOCK[1]

A. J. RIKER AND G. W. KEITT[2]

The writers[3] recently reported that extensive isolation studies had failed to reveal the presence of *Bacterium tumefaciens* Smith and Town. in overgrowths on certain apple trees which had been rejected at nurseries because of supposed crowngall infection. They suggested the working hypotheses: (a) that the malformations dealt with on these trees were not induced by the crowngall organism; (b) that their development was merely incidental to the root-grafting method employed in the propagation of this stock. Further studies have yielded results which are in full conformity with these hypotheses.

The isolation studies previously reported have been extended to 227 apple trees which were representative of lots of stock sent in as crowngall rejects by twelve nurseries in six states. Less than two per cent of these trees yielded *Bact. tumefaciens*. The overgrowths which yielded the crowngall organism were of the "soft gall" type. Tests of the isolation technique on known crowngall at frequent intervals throughout the period of experimentation gave positive results from 44 of 46 plants studied.

A study of the trees which failed to yield *Bact. tumefaciens* showed that most of the malformations developed immediately above places where there was interference with the downward passage of elaborated food. The most common cause of this stoppage was the failure of some part of the cion to unite with the stock. The effects produced appear to be closely comparable with those which follow girdling. Many of the trees, especially those one year old, showed clearly that the failures of union were the result of poor fits in grafting. An examination of representative freshly prepared grafts from seven of the nurseries from which stock had been secured for the isolation experiments showed ample numbers and types of misfits to account for the abnormalities found. This situation is not surprising in view of the fact that in many nurseries individual workmen cut and fit as many as three thousand grafts a day, an average of one in about twelve seconds of working time.

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[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] This work, which is being administered by the Crop Protection Institute, is supported cooperatively by the American Association of Nurserymen and individual nurserymen, the Iowa State College of Agriculture and Mechanic Arts, and the University of Wisconsin, in collaboration with the U. S. Department of Agriculture. Coordinated research programs are in progress at the Iowa State College of Agriculture and Mechanic Arts and at the University of Wisconsin.

[3] Riker, A. J., and G. W. Keitt. A report of progress on studies of crowngall in relation to nursery stock. Science, N. S., 62: 184–185. 1925.

A study has been made of the types of misfit found in freshly prepared piece-root apple grafts selected at random at certain large nurseries, and plantings have been made for the purpose of following the development of malformations in relation to fit.

Overgrowths similar to those found on the rejected nursery stock studied were produced at will on two-year-old apple trees by certain types of wounding. Likewise, in experiments in which precautions were taken to exclude *Bact. tumefaciens,* malformations about the unions of piece-root apple grafts have been induced or suppressed almost at will by variations in the technique of grafting.

These and other results appear to justify the conclusions: (a) that the major portion of the overgrowths encountered on the piece-root apple grafts thus far studied by the writers, developed incidentally to the grafting process, without the intervention of *Bact. tumefaciens;* and (b) that the occurrence and development of such malformations may be reduced by modifications in grafting practice. It remains for further investigations to determine the relative prevalence of true crowngall and of these non-parasitic overgrowths on apple root grafts under different regional and seasonal conditions, and to show to what extent the latter types of malformation may be controlled in commercial propagation by modifications in grafting practice. Studies bearing on these and related questions are being continued.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN

PHYTOPATHOLOGICAL NOTES

Crown Wart of Alfalfa in Indiana. On June 12, 1925, while examining alfalfa fields near Madison, Indiana, the writer found a number of plants in two fields attacked by what appeared to be crown wart. Microscopical examination of galls from these fields showed that typical fruiting bodies of *Urophlyctis alfalfae* were present in abundance. The one field had been sown some twenty years ago and still had about a fourth of a stand of plants fairly uniformly distributed. No positive evidence was obtained that the disease was causing any damage. However, the season had been very dry and the first cutting had just been made; hence this was not an opportune time for making observations.

As far as the writer has knowledge, this is the first report of the occurrence of this disease east of the Rocky Mountains.—J. L. WEIMER, U. S. Dept. of Agriculture, Washington, D. C.

The Avocado Scab Organism. It has been generally accepted that the fungus causing citrus scab also produces the disease of avocado commonly known as avocado scab. Repeated artificial field inoculations on avocado with the citrus scab organism, however, have failed to show infection; while similar inoculations on avocado with the fungus associated with avocado scab have reproduced the disease in all cases. A critical study of the fungus from avocado indicates that it is an undescribed species of the form-genus *Sphaceloma.* A technical description of the new species is being prepared in connection with an account of its pathology.—ANNA A. JENKINS, Bureau of Plant Industry, U. S. Department of Agriculture.

Browning Disease of Flax in North America. An important disease of flax, characterized by brown lesions on stems, leaves, and bolls, and a tendency of certain of the diseased stems to break over near the ground line, was described by Lafferty in Ireland in 1921. The former symptoms were referred to as "browning" and the latter as "stem break," and both were shown to be caused by the same organism. Affected stems are brittle, and break during scutching, resulting in shortened fibers; and the yield and quality of the seed also are reduced. The pathogene was described as *Polyspora lini* Lafferty, a new genus and species.[1]

This disease has been present in North America at least since 1920. In August of that year the writer noticed a peculiar spotting on several varieties of flax in the Field Husbandry experimental plots of the University of Saskatchewan, Saskatoon, Saskatchewan, Canada. Specimens were collected, but no attempt was made at that time to isolate the causal organism. In 1923, "stem break" caused by *Polyspora lini* was reported by Fraser[2] as severe in two one-fortieth acre plots at Saskatoon. The writer again collected the disease from varietal plots at Saskatoon in August of the same year, and isolated the causal organism several weeks later. The following fall, *Polyspora lini* was isolated from some of the diseased leaves collected in 1920. These had been kept dry in the laboratory. The fungus had therefore remained alive in this condition for more than four years. Pethybridge *et al*[3] found spores of the fungus to survive on dry flax seed for two and one-half years, but not three years. He points out that infected seed readily transmit the disease to the following crop. The pathogene may therefore be readily introduced into new localities by infected seed.

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[1] Lafferty, H. A. The browning and stem break disease of cultivated flax (*Linum usitatissimum*), caused by *Polyspora lini.* Sci. Proc. Roy. Dub. Soc. n.s. 16: 248–274. 1921.

In August, 1925, specimens of diseased flax were received from Mr. B. B. Robinson, of East Lansing, Michigan. These specimens were collected from a field about 100 miles north of East Lansing, in which about one per cent of the plants were affected. Part of the seed from which this crop was grown was obtained from Ontario, Canada. Two diseases were found on these plants, namely, rust (*Melamspora lini*) and "browning" (*Polyspora lini*). The latter fungus was isolated, and cultures and lesions on the stems compared with authentic material received from Ireland and with that from Saskatoon and found to agree. No previous record of the occurrence of "browning" in the United States has come to the writer's attention.

The symptoms thus far observed have been typical of the "browning" stage of the disease, rather than the breaking over of the plants characteristic of "stem break."

An organism isolated from diseased flax collected at Crookston, Minnesota, and St. Paul, Minnesota, in 1924, resembles *Polyspora lini*, but is not identical with it. It was reisolated from flax which had been artificially inoculated in the field in 1925.—A. W. HENRY, University of Minnesota.

Personals.—Dr. Perley Spaulding, Pathologist of the Office of Investigations in Forest Pathology, Bureau of Plant Industry, Washington, D. C., has been assigned to duty as Pathologist with the Northeastern Forest Experiment Station, at Amherst, Mass. Correspondence and exchanges should be thus addressed.

[2] Survey of the Prevalence of Plant Diseases in the Dominion of Canada. Fourth Annual Report. p. 85. 1923.

[3] Pethybridge, G. H., H. A. Lafferty, and J. G. Rhynehart. Investigations on flax diseases. Jour. Dept. Agr. and Tech. Instruction for Ireland 21: 9. 1921.

Dr. W. D. Moore, Assistant Pathologist in Extension Work, Clemson Agricultural College, South Carolina, has been granted a year's leave of absence and will spend the time as Pathologist for the Truckers Supply Co., Beaufort, South Carolina. He expects to do research work on some of the diseases of truck crops.

Ellsworth Bethel, the well known uredinologist, died at Denver, Colorado, September 8, 1925, aged 62 years.

The Department of Plant Pathology of the Virginia Polytechnic Institute has been expanded to include botany and is now designated as the Department of Botany and Plant Pathology. The Department of Biology, which formerly included botany, has been discontinued, following the resignation and retirement of Dr. E. A. Smyth, Jr., professor of biology. The organization of the enlarged Department is as follows: professor, F. D. Fromme; associate professors, H. S. Stahl (instruction), A. B. Massey (instruction), S. A. Wingard (research); assistant professors, F. J. Schneiderhan (research), James Godkin (extension); instructor, R. H. Hunt (research); assistant, C. N. Priode (research).

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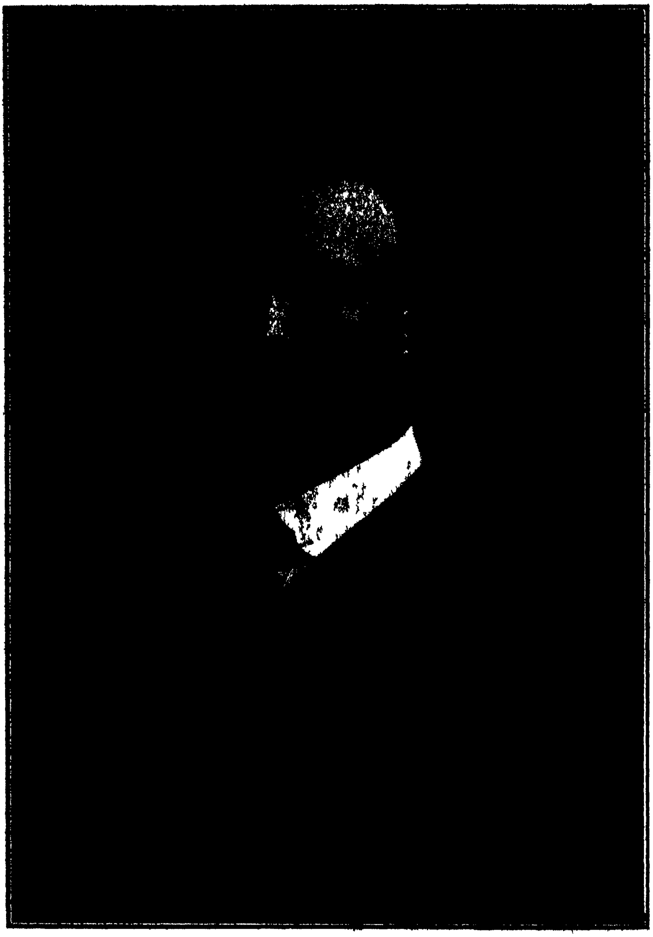

AUGUSTINE DAWSON SELBY

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W. G. STOVER

WITH PORTRAIT, PLATE I

Augustine Dawson Selby, sometime President of the American Phytopathological Society, and for nearly thirty years Botanist of the Ohio Agricultural Experiment Station, died at Wooster, Ohio, May 7, 1924.

He was born near Sharpsburg, Athens County, Ohio, September 2, 1859. He grew up on the farm and retained throughout his life a practical interest in the difficulties and problems confronting the grower of plants, whether on the farm, in the orchard or in the greenhouse. He was married December 15, 1883, to Miss Libbie Glover, of Hilliards, Ohio. She and an only son, Warren F. Selby, an attorney at Akron, Ohio, survive.

In his younger manhood he taught in the public schools for several years. He was superintendent of schools at Huntington, W. Va., and principal of schools at Ironton and Columbus, Ohio. He began his life's work in botany as a teacher of that subject in the Columbus High School from 1889 to 1894. During this period he also carried on collegiate studies at Rutgers College and at Ohio State University, receiving from the latter the degree of Bachelor of Science in 1893.

At these schools he came in contact with two men who greatly influenced his further scientific work. Dr. B. D. Halsted had, in 1889, entered upon his long career at the New Jersey institution, and had published several of the papers on plant diseases for which he became noted among the botanists of America. Dr. W. A. Kellerman, who had begun at the Kansas Agricultural College his important work on cereal diseases, especially the smuts and their control, was continuing these and other mycological and pathological studies in Ohio. Professor Selby also studied at Washington University and the Shaw School of Botany in 1899, and at Columbia University in 1902–3.

In September, 1894, he assumed his duties at the Ohio Agricultural Experiment Station which had been moved from the campus of Ohio State University to Wooster in 1892. Although the station had been established twelve years earlier, its resources were meager and its staff was small. Professor Selby, as he was familiarly known to his associates and to thousands of Ohio farmers, undertook and developed the station work in the two important lines of botany and chemistry. In 1902, however, a separate department of chemistry was established, and Professor Selby gave his time during the remainder of his active life to studies in botany, plant pathology and plant breeding.

A study of oats smut and its prevention (1895) was the first of a long series of bulletins and circulars bringing to the attention of Ohio farmers the results not only of his own experiments and observations but also those of scientific workers in other states and in foreign countries. In successive bulletins he summarized in an able manner the scientific knowledge of the day on the diseases of the peach, cucumber, muskmelon, tomato, tobacco, wheat and other crop plants, always including valuable suggestions from his own observations or investigations. When we consider the meager knowledge concerning plant diseases available to the farmer at that time, it appears impossible to overestimate the value of these contributions. Of a similar character, on a larger scale, is "A condensed handbook of the diseases of cultivated plants in Ohio" (1900) which was later revised and extended (1910). This handbook was, at the time of its publication, the only general compilation of the American aspects of phytopathology. In consequence, it was widely used outside of Ohio and was, in fact, for nearly a decade the standard text book on the subject.

His work as Station Botanist was in large part practical in nature. He conducted extensive experiments on spraying the peach, grape, tomato, cucumber, muskmelon and other plants in various parts of the state, and on seed treatment of wheat, oats and potato, continuing the work until definite results were obtained in each case He was the first to suggest the use of the formaldehyde drip method for the control of onion smut, and was the first to report the occurrence in America of *Thielavia basicola* (B. and Br.) Zopf.

In addition to his work on plant diseases he gave considerable attention to the native plants and the weeds of Ohio. Besides a number of shorter papers he published "A first Ohio weed manual" (1897) and "A second Ohio weed manual" (1906). Several seasons (1897–1900) were occupied in part with sugar-beet investigations, and for a number of years following 1903 he supervised the work on tobacco breeding at the Germantown substation in the heart of the Miami Valley tobacco section.

During his later years, Professor Selby's health was not of the best, and he limited himself more to administrative and office duties, although he continued his visits to growers in various parts of the state as necessary. On several occasions he expressed to the writer his desire to remain "in the harness" as long as possible in order that he might continue to have some mental occupation. He retired July 1, 1923.

Dr. Charles E. Thorne, Director of the Ohio Agricultural Experiment Station for many years, writes of him:

"My first acquaintance with Mr. Selby was when, as a young student at the Ohio State University, he applied for employment on the University farm. I have always carried, in the picture gallery of my memory, a view of his pleasant address when we first met, and when, in 1894, his name was suggested by my coadjutor, W. J. Green, for the position of Botanist and Chemist at the Experiment Station I was glad to invite him to that position.

"At that time the Station had just been relocated in Wayne County, a relocation involving serious differences of opinion and tedious legislation, and its resources were not such as to justify the employment of separate heads for these two important lines of work, but Mr. Selby entered upon the work with enthusiasm and ability, soon calling young assistants to his aid, and thus he carried the work forward until increasing support of the Station's work made it possible to release him from responsibility for the chemical work and concentrate his energies upon his favorite studies in Botany and Vegetable Pathology.

"Born and reared on an Ohio farm, a farm of which he eventually became the owner and possession of which he retained through life, he always maintained a sincerely appreciative interest in the farmers' problems, and his publications on the control of weeds and plant diseases were prepared with the actual conditions of the farm always in view.

"He was constantly on the lookout for new dangers threatening the interests of the farm in his field of work, and was always ready to devote his energies to their control.

"His health was never perfect, and he spent parts of several winters on leaves of absence in southern climates—Italy, Cuba, the Isle of Pines, and South America.

"His church affiliations were with the Episcopalians, and he belonged to the Masonic order."

Professor Selby began his work on plant diseases during the pioneer epoch of American phytopathology, before the organization of the large graduate departments of plant pathology in our universities and before the development of much of the technique of the present-day plant disease laboratory. Much that we now rightly consider elementary information was then unknown. He played no small part in bringing the science to its present development and especially in carrying the results of research and experiment to the farmers of his state, notwithstanding the fact that his work was often limited by lack of funds.

Professor Selby was one of the earliest and most persistent advocates of the organization of the American Phytopathological Society, and took a very active part in the stormy discussions that attended its birth. To its organization he contributed freely of both time and money, and no one was more gratified than he at the rapid growth of the Society and particularly at the success of its journal. He had infinite faith in the future of plant pathology, and confidently looked forward to the day when there would be "a plant pathologist in every county."

Professor Selby was a Fellow of the American Association for the Advancement of Science and member of the Council in 1911; a member of the Botanical Society of America; a member of the American Phytopathological Society (one of the charter members) and its President in 1911; a member of the Torrey Botanical Club; a charter member of the Ohio State Academy of Science and its President in 1901; a member of the St. Louis Academy of Sciences; a member of the American Genetic Association, and a member of the Ohio State Horticultural Society.

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1888

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1889

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1891

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1896

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THE DILOPHOSPORA DISEASE OF CEREALS

D. ATANASOFF

WITH NINE FIGURES IN THE TEXT

INTRODUCTION

The disease here under consideration was seen for the first time about a century ago (9). Since then it has been repeatedly observed on various cereals and grasses and has often caused alarm among agriculturists. Most of the numerous short notes and papers on this subject describe briefly the fungus associated with the disease under the incorrect names of *Dilophospora graminis* Desm. and *Dilophia graminis* Fuckel, while some of them contain also short and incomplete descriptions of the malady itself. No study of the organism, its physiology and pathogenicity has ever been made. The disease, the way in which it develops and the conditions under which it occurs have also never been investigated.

The writer observed this disease for the first time during the summer of 1923 in a field sown with a mixture of wheat, spelt and rye. The symptoms and effects of the disease were so striking that the diseased plants attracted attention from a distance. A large number of plants were already dead. Others were very much deformed, while some of the apparently normal plants had partially or wholly deformed and charred heads.

The presence of numerous pycnidia over all infected areas indicated the fungous nature of the disease. A careful examination of the deformed heads, however, showed in all cases also the presence of numerous nematode galls which were filled with living larvae of *Tylenchus tritici* (Steinbuch) Bastian, the cause of the nematode disease of cereals. The presence of the nematode galls in all infected heads was so constant that it suggested at once that the occurrence of the two diseases simultaneously on the same plants can not be considered a mere coincidence. From the literature on this disease it became evident that the same condition has been observed also by other workers. In view of this it seemed desirable to plan and carry out the experimental work on this problem from the very beginning in connection with the nematode disease.

The two principal questions which had to be answered were: 1. Is *Dilophospora alopecuri* (Fr.) Fr. a pathogene and in position to bring about alone the disease on wheat, spelt, and rye as observed in nature; and 2. Do the nematodes and the disease, which they cause, favor the parasitism of Dilophospora on the same plants.

All of the numerous experiments made in order to answer the first question gave absolutely negative results, in spite of the fact that infection experiments were made with wheat, spelt, and rye of various ages and of the same varieties on which the disease had been observed; soil, seed, and wound infections at various moistures and temperatures of soil and air were also made but the results were in all cases negative.

The answering of the second question was much easier. In numerous infection experiments, made with several thousands of plants, in all cases without exception, typical and abundant symptoms of the Dilophospora disease appeared on all plants simultaneously infected also by the nematode disease. The appearance of the symptoms of the nematode disease preceded in all cases the appearance of the symptoms of the Dilophospora infection, sometimes within a few days, sometimes with longer periods of time.

The experimental results, negative and positive, showed that while *Dilophospora alopecuri* is a good parasite, or at least a very destructive and injurious fungus, it can attack and establish itself successfully only on the plants already suffering from the nematode disease, and only as long as the nematodes are present in the plants. The further spread of the fungus attack stops as soon as the nematodes leave the plants, die, or form galls. What the nematode disease fails to destroy is destroyed by the Dilophospora disease; this it does with remarkable rapidity and thoroughness.

The further investigations showed not only that the pathogenicity of *D. alopecuri* is influenced by the nematode disease, but also that the relation of the two pathogenes is a more complicated one: not only that the nematodes evidently make the plants susceptible to *D. alopecuri* but they are also an important carrier of the spores of this fungus. And what is even more important they, while creeping and crawling between the leaf sheaths on their way to the interior, the growing point, and later to the young head of the plant, take along the spores of the fungus and deposit them on the growing point of the same. Reaching the growing point of the plant by the spores of the fungus, which seems essential for its successful parasitism, can not be accomplished without the nematodes.

For the nematodes this co-parasitism is not only of no advantage but decidedly harmful. Indeed *D. alopecuri* is, as will be shown later, a most important factor in checking the nematode disease in nature.

A co-parasitism of this degree, the absolute dependence of the one parasite upon the second one, and the effective indirect extermination of the second parasite by the first, is for vegetable pathology unique. A further study of this and related cases, however, might show that this phenomenon plays a greater rôle in nature than we now suppose.

The very dependence of the Dilophospora disease on the nematode disease, for which there exist reliable and efficient control measures, makes the controlling of the first disease an easy matter, since controlling the nematode disease means also controlling the Dilophospora disease.

GEOGRAPHIC DISTRIBUTION OF THE DISEASE

The Dilophospora disease, though not generally known, is quite widely distributed. In Holland the writer so far has observed the disease on several fields near the village of Epen, situated on the Belgian border. Besides Holland (36, p. 28) it has been reported in the course of time on numerous grasses and cereals from various places in France and Germany and occurs occasionally on wild grasses (1) and wheat in England.[1] The disease has been observed also repeatedly in Norway, Denmark, and Switzerland. It has been reported also from Servia (33, p. 398). Bessey (2) observed the disease on *Calamagrostis canadensis* plants gathered in Kenosha County, Wisconsin, U. S. of America.

HOSTS

A large number of plants are attacked by this disease, but all of them belong to the grass family—the Gramineae. Whether the Dilophospora, which has been observed as a parasite on many grasses and cereals, belongs to *D. alopecuri*, the only species described so far, is not at all certain and not very likely. As long as this is not established experimentally the list of host plants given below will be of a provisional character. The plants listed below have been reported as hosts of *D. alopecuri* since the establishment of this genus and species in 1840 (5) and 1828 (9), respectively.

The writer observed the disease only on wheat, rye and spelt in about the same proportions. Infection experiments with these plants gave in all cases 100 per cent of infected plants, while infection experiments with barley and oats so far have given negative results.

In the table below are also given the names of the workers who have observed for the first time the occurrence of Tylenchus galls on the respective host plants. This table shows also very plainly the existence of a certain relation between the Dilophospora and nematode diseases.

ECONOMIC IMPORTANCE

Though there are practically no data regarding the economic importance of this disease its occurrence has often caused great alarm among the agriculturists of various countries. Berkeley (1) writes of this disease that: "In a field of seven acres a fourth of the wheat was diseased to such a degree that many of the ears were altogether abortive, while in the best there were only two or three tolerable grains." Richon (34) writes that in some

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[1] According to a letter of Dr. Cotton, Kew, England.

TABLE 1—*First reports of Tylenchus upon the listed host plants. Also the reported occurrence of Dilophospora alopecuri*

Number	Name of host plants	*Dilophospora alopecuri* (Fr.) Fr.		*Tylenchus tritici* (Steinbuch) Bastian or other gall forming nematodes	
		Author	Date	Author	Date
1	*Agrostis sp.*	Desmazières	1840		
2	*Agrostis alba*	Kirchner	1906	Hieronymus	1890
3	*Agrostis vulgaris*	Kirchner	1906	Schlechtendal	1885
4	*Alopecurus agrestis*	Fries	1828		
5	*Alopecurus pratensis*	Sorauer	1874		
6	*Arrhenatherum elatius*	Kirchner	1906		
7	*Calamagrostis canadensis*	Beesey	1906	Bessey	1906
8	*Calamagrostis epigeios*	Diedecke (Jaap)	1907		
9	*Dactylis glomerata*	Kirchner	1890	Jahresber. dan. Samenkontrollstation	?[1]
10	*Festuca ovina*	Karsten	1865	Schlechtendal	1885
11	*Festuca pratensis*	Kirchner	1906		
12	*Festuca rubra*	Kirchner	1906	Jahresber. dän. Samenkontrollstation	?[1]
13	*Phleum pratense*	Rostrup	1905	Hieronymus	1890
14	*Holcus lanatus*	Fuckel	1861	Ritzema Bos	1900
15	*Holcus mollis*	Desmazières	1840		
16	*Secale cereale*	Desmazières	1840	Roffredi	1776
17	*Triticum spelta*	Stormer	1904	Stormer	1904
18	*Triticum vulgare*	Berkeley	1862	Needham	1747

of the fields examined by him the heads and culms infected represented 5 per cent of the yield. Pape (30) states that "this year in Baden one case was observed where part of a field showed 30 per cent infection, so that the occurrence of the disease caused great anxiety in the territories concerned." Kessler (19) on the other hand writing of same cases states that "in judging the injury it should be kept in mind that in general the heads of only 5 per cent of all infected plants come out from the leaf sheath and attain nearly the height of the healthy heads. By far the greatest majority of the infected plants are overlooked by a superficial examination of the fields, so that the estimated loss is usually too low. Only a careful examination discloses the numerous dwarf plants with strongly misformed heads and dead

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[1] According to Reh in Sorauer's Handbuch der Pflanzenkrankheiten 3:29. 1913.

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